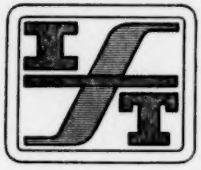
Food Research

An Official Publication of

The Institute of Food Technologists



IN THIS ISSUE

The Effect of Selected Pretreatments Upon the Culinary Qualities of Eggs Frozen and Stored in a Home-Type Freezer. I. Plain Cakes and Baked Custards

Fatty Acids of the Lipids From Freshly Canned Florida Valencia Orange Juice

Effect of Blanching and of Frozen Storage of Vegetables on Ascorbic Acid Retention and on the Concomitant Activity of Certain Enzymes

The Stability of Thiamine, Riboflavin, and Nicotinic Acid of Lamb Chops During Frozen Storage and Subsequent Cooking

Ascorbic Acid Content of Some Miscellaneous Tropical and Subtropical Plants and Plant Products

Role of Pectin as Related to Dehydration and Rehydration of Simulated Fruit Preparations

The Antioxidant Properties of Natural Spices

Thiamine, Riboflavin, and Niacin Content of Processed Meats

Influence of Carbohydrate and Nitrogen Sources Upon Lipid Production by Certain Yeasts

The B Vitamins in Raw and Cooked Lamb. I. Thiamine

The Effect of Particle Size on the Nutritional Characteristics of Unifine Flour

Effects of Various Physical Treatments Upon the Amyloclastic Susceptibility of Starch

Volume 17 **JANUARY-FEBRUARY, 1952** **Number 1**

Food Technology

Published Monthly by the

INSTITUTE OF FOOD TECHNOLOGISTS

The Companion Journal to

FOOD RESEARCH

If you are a reader of FOOD RESEARCH your library is incomplete without FOOD TECHNOLOGY—devoted to the publication of the results of research and their practical application in industry. Contributors to its pages represent many fields of scientific and technological interest.

SUBSCRIPTION RATES

FOOD RESEARCH	$ 5.50 Domestic	$ 6.00 Foreign
FOOD TECHNOLOGY . . .	7.50 Domestic	8.50 Foreign
Both Journals	12.00 Domestic	13.50 Foreign

Send Subscriptions to

FOOD TECHNOLOGY
119-123 West Park Avenue
CHAMPAIGN, ILLINOIS

Food Research

An Official Publication of
The Institute of Food Technologists

Volume 17 JANUARY-FEBRUARY, 1952 Number 1

EDITORIAL BOARD

Z. I. KERTESZ, *Editor-in-Chief*
New York State Agricultural Experiment Station
Geneva, New York

A. K. BALLS
J. S. BLAIR
G. M. DACK
ROBERT S. HARRIS
L. B. HOWARD
L. B. JENSEN
M. A. JOSLYN
A. G. LOCHHEAD
PAULINE PAUL
CATHERINE J. PERSONIUS
SAMUEL C. PRESCOTT
J. J. WILLAMAN

FRED W. TANNER, *Editor Emeritus*

CHARLES S. LAWRENCE, *Business Manager*
176 West Adams Street
Chicago 3, Illinois

Published by the Institute of Food Technologists six times a year. Printed by The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois. Entered as second-class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879.

Why these foods have **PLUS VALUE**

With today's big interest in food values, Mrs. Homemaker wants not only good-tasting foods—but foods which she knows are good for her family.

That's why these good foods have been made better, through enrichment. These great grain foods are *dynamic*—because they are rich in energy-producing substances . . . dynamic because of their enormous public acceptance . . . dynamic because they are enriched to overcome the unavoidable loss of important vitamins and minerals which occurs during production.

Please remember this: the quality of the foods you process is vital to your success. In judging the quality of grain foods, it is most important to consider the plus value given to them by enrichment.

VITAMIN DIVISION • HOFFMANN-LA ROCHE INC. • NUTLEY 10, N. J.

Volume 17 JANUARY-FEBRUARY, 1952 Number 1

CONTENTS

THE EFFECT OF SELECTED PRETREATMENTS UPON THE CULINARY QUALITIES OF EGGS FROZEN AND STORED IN A HOME-TYPE FREEZER. I. PLAIN CAKES AND BAKED CUSTARDS. Ruth Jordan, Betty Noble Luginbill, L. E. Dawson, and C. J. Echterling 1

FATTY ACIDS OF THE LIPIDS FROM FRESHLY CANNED FLORIDA VALENCIA ORANGE JUICE. Lyle James Swift 8

EFFECT OF BLANCHING AND OF FROZEN STORAGE OF VEGETABLES ON ASCORBIC ACID RETENTION AND ON THE CONCOMITANT ACTIVITY OF CERTAIN ENZYMES. Eva R. Hartzler and N. B. Guerrant 15

THE STABILITY OF THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID OF LAMB CHOPS DURING FROZEN STORAGE AND SUBSEQUENT COOKING. W. P. Lehrer, Jr., A. C. Wiese, W. R. Harvey, and P. R. Moore 24

ASCORBIC ACID CONTENT OF SOME MISCELLANEOUS TROPICAL AND SUBTROPICAL PLANTS AND PLANT PRODUCTS. Margaret J. Mustard 31

ROLE OF PECTIN AS RELATED TO DEHYDRATION AND REHYDRATION OF SIMULATED FRUIT PREPARATIONS. George L. Baker, John F. Kulp, and Ralph A. Miller . 36

THE ANTIOXIDANT PROPERTIES OF NATURAL SPICES. J. R. Chipault, G. R. Mizuno, J. M. Hawkins, and W. O. Lundberg 46

THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF PROCESSED MEATS. B. S. Schweigert, Barbara A. Bennett, Mona Marquette, Harold E. Scheid, and B. H. McBride 56

INFLUENCE OF CARBOHYDRATE AND NITROGEN SOURCES UPON LIPID PRODUCTION BY CERTAIN YEASTS. S. S. Husain and M. M. Hardin 60

THE B VITAMINS IN RAW AND COOKED LAMB. I. THIAMINE. Ethelwyn B. Wilcox and Leora S. Galloway 67

THE EFFECT OF PARTICLE SIZE ON THE NUTRITIONAL CHARACTERISTICS OF UNIFINE FLOUR. Mary Corbett Stevens, Janice F. Sieburth, Tom Wahl, and Barbara McLaren 74

EFFECTS OF VARIOUS PHYSICAL TREATMENTS UPON THE AMYLOCLASTIC SUSCEPTIBILITY OF STARCH. Frances E. Volz and P. E. Ramstad 81

SUBSCRIPTION RATES

To members of the Institute of Food Technologists, $4.50 per volume.

Non-members: Domestic, $5.50 per volume; foreign, $6.00 per volume.

Subscriptions and renewals are entered to begin with the first issue of the current volume.

Single copies of current volume: Domestic, $1.00; foreign, $1.15.

CORRESPONDENCE

Editorial: Z. I. Kertesz, Editor-in-Chief, New York State Agricultural Experiment Station, Geneva, New York.

Non-Member Subscriptions, Back Volumes, Single Copies, and Reprints: FOOD RESEARCH, 119-123 W. Park Ave., Champaign, Ill.

Members Subscriptions: THE INSTITUTE OF FOOD TECHNOLOGISTS, 176 W. Adams St., Chicago 3, Ill.

Advertising: Charles S. Lawrence, Business Manager, 176 W. Adams St., Chicago, Ill.

CLAIMS

Claims for copies lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

NOTE

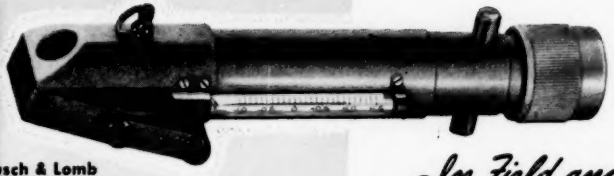
The Institute of Food Technologists assumes no responsibility for statements in articles appearing in FOOD RESEARCH. The opinions expressed do not necessarily reflect policies of the Institute of Food Technologists.

Manuscripts for Food Research should conform to the style used in the journal and be submitted in duplicate to the Editor-in-Chief. Authors of papers intended for FOOD RESEARCH should request from the Editorial Office a copy of the booklet "Information and suggestions for authors." FOOD RESEARCH reserves the privilege of editing manuscripts to make them conform with the adopted style of the journal or returning them to the author for revision. Changes made by the Editorial Office may be reviewed by authors when they examine proof.

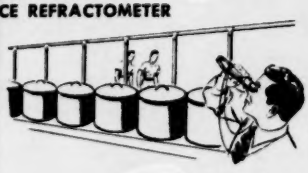
Copyright, 1952
By the Institute of Food Technologists
Printed in U. S. A.

Use Both REFRACTOMETERS and . . .

You Can't Be Wrong . . .



Bausch & Lomb
JUICE REFRACTOMETER



Bausch & Lomb
"ABBE-56"
REFRACTOMETER

. . . In Field and Plant Checks

In field, vineyard, orchard or grove . . . on plant production lines . . . *on-the-spot* checking for maturity or quality is easy, quick and accurate with the Bausch & Lomb Juice Refractometer. Here, *for the first time,* is a hand refractometer that uses either reflected light for clear solutions, or transmitted light for dense, opaque or turbid samples. Quick, *direct* readings, 0.1% accuracy, with a range of 0 to 25% dissolved solids. Adjustable eyepiece, for individual comfort.

. . . In Laboratory Control

For laboratory quality control . . . for research . . . you get highest-accuracy testing of transparent and opaque liquids and solids, by either transmitted or reflected light, with the B&L "Abbe-56"—*the world's finest Abbe-type refractometer.* The illuminated glass scale, with extra-wide field, provides a *clear* view of *three* scale numbers at all times. Index range is 1.30 to 1.71; theoretical accuracy, ±.0001. Scale and all internal moving parts are protected from splashing, fumes and shock by a sturdy metal housing. An easily interchangeable spare prism set (optional) ends down time. You save time, cut costs and increase operator comfort and efficiency with the B&L "Abbe-56."

Write for complete information and a demonstration to Bausch & Lomb Optical Co., 765-14 St. Paul St., Rochester 2, N. Y.

Bausch & Lomb REFRACTOMETERS

THE EFFECT OF SELECTED PRETREATMENTS UPON THE CULINARY QUALITIES OF EGGS FROZEN AND STORED IN A HOME-TYPE FREEZER.

I. PLAIN CAKES AND BAKED CUSTARDS[a,b]

RUTH JORDAN, BETTY NOBLE LUGINBILL,[c] L. E. DAWSON,[d] AND C. J. ECHTERLING

Departments of Home Economics and Poultry Husbandry, Agricultural Experiment Station, Purdue University, Lafayette, Indiana

(Manuscript received April 3, 1951)

Frozen eggs have been produced on a commercial scale for some time and are widely used in the food manufacturing industries *(16)*. On the other hand, they have not been generally available to the home user. Schaible and Card *(10)* suggested a method of freezing eggs in thin bars to be sold at retail for household use, but, so far as is known, this method has not been used on any large scale.

With the increase in the number of frozen food locker plants and of home freezers, the feasibility of preparing and storing frozen eggs for home use has increased. Stewart *(11)* recommended that farm families, particularly, should freeze eggs when they are plentiful and store them in frozen food lockers for later use.

Since eggs are a very perishable product, care must be exercised to avoid bacterial contamination. The sanitary measures necessary to produce high quality frozen eggs have been summarized by Winter and Wrinkle *(18)*. In recent years, experiments have been made on the pasteurization of liquid egg prior to freezing *(8, 20)*.

The functional properties of egg whites are little affected by freezing and thawing, but untreated frozen yolks when defrosted may be watery and ropy *(15)*, rubbery and gummy *(16)*, or become a pasty, viscous gel which does not combine well with other ingredients in food preparation *(6)*. To a lesser degree, the same changes may occur in frozen whole egg magma. In order to overcome these unfavorable effects, commercial producers frequently treat the egg yolk or mixed whole egg with salt, sugar, sirup, or glycerine, or subject it to homogenization before freezing. A few studies on the effectiveness of these treatments have been reported *(9, 13, 15)*.

Frozen eggs have been found to be satisfactory for use in cakes, custards, and other cooked products *(5, 8, 19)* and numerous publications *(1, 2, 7, 14)* have appeared which give directions for preparing, packaging,

---

[a] A part of this article is based upon a thesis written by Betty Noble Luginbill in partial fulfillment of the requirements for the degree of Master of Science in Home Economics at Purdue University in February, 1949.

[b] Journal Paper No. 530, Purdue University, Agricultural Experiment Station.

[c] Present address: Bozeman, Montana.

[d] Present address: Michigan State College, East Lansing, Michigan.

and freezing eggs for home use. In most of these directions, the addition of varying proportions of salt, sugar, sirup, or honey to the egg yolks or mixed whole egg is advised. Information seems to be lacking with regard to the effect of these recommended treatments upon the functional properties of the eggs after various periods of frozen storage in a home-type freezer.

The chief objective of this investigation was to determine the effect of selected pretreatments upon the culinary qualities of egg yolk and mixed whole-egg frozen and stored in a home-type freezer and to observe the keeping quality of the eggs so treated. Plain cakes and baked custards were used in this phase of the study.

EXPERIMENTAL PROCEDURE

Eggs and their preparation. The eggs used for freezing were secured from a flock of 56 hens of the Purdue Poultry Farm's strain of New Hampshires. These hens were selected on the basis of the quality and uniformity of their eggs. The average VanWagenen-Wilgus *(17)* score for the eggs laid by the hens was about 2.0 with a range of 1.5 to 2.5, and the Haugh *(3)* unit value of the eggs averaged about 92 with a range of 80 to 104. The hens were housed in individual laying cages. The eggs were gathered twice daily and held in a cooler at a temperature of approximately 45°F. (4.5°C.) until broken out. Since for the egg-yolk custards more eggs were needed for freezing than could be produced by the special flock at one time, it was necessary to supplement the eggs with graded eggs from other flocks. These eggs were scored as they were broken out, and any eggs scoring higher than 3.0* by the VanWagenen-Wilgus chart were discarded. The eggs for the three replications were obtained between March 22 and April 9; thus they were laid during the season of normal high production.

In preparation for freezing, the broken-out whole eggs were cautiously stirred to break the yolk membranes and then carefully mixed to blend yolks and whites with as little incorporation of air as possible. The mixed eggs were strained through a wire-mesh household strainer to remove chalazas, then weighed into portions for the seven treatments. Likewise, the egg yolks to be frozen were carefully mixed and strained with care taken to avoid incorporation of air.

The treatments given the seven portions of yolks or of whole-egg magma before freezing were as follows: To one portion nothing was added; to the second and third portions, respectively, table-grade salt was added in the proportion of 1 teaspoon (6 g.) and 2 teaspoons (12 g.) per cup (243 g.) of egg; to the fourth and fifth portions, respectively, granulated sugar was added in the proportions of 1 tablespoon (12.5 g.) and 2 tablespoons (25.0 g.) per cup of egg; and to the sixth and seventh portions, respectively, white corn sirup was added in the proportions of 1 and 2 tablespoons (20.5 and 41.0 g., respectively) per cup. The added ingredient was thoroughly mixed with the egg magma.

Small waxed paper cartons (3-ounce and 4-ounce sizes) with close-fitting lids were used for packaging the eggs. Into each carton was weighed enough of the egg mixture for one baking of custard or plain cake as the case might be. The packaged eggs were placed in a home-type freezer, frozen and stored at about 0°F.(−18°C.).

Throughout the preparation and packaging of the eggs, care was taken to avoid contamination or exposure to warm temperatures. While one portion was being mixed, weighed, and packaged, other portions were kept in the refrigerator. The first samples for use in baking tests were withdrawn after 1 week of frozen storage; subsequent bakings were made after the eggs had been in frozen storage for 2, 4, and 6 months.

Each time frozen samples were used for baking tests, fresh shell eggs from the same flock (except in a few cases) were used for making control samples. Thus, for the study, there were 8 treatments (1 fresh shell egg sample and 7 frozen samples),

*By the VanWagenen-Wilgus score, eggs scoring between 1.0 and 3.0 are Grade A quality or better; eggs scoring higher than 3.0 are of lower quality.

4 storage periods, and 3 replications; consequently, for each type of product made, a total of 96 units was baked.

Plain cakes. The basic formula for the cake was one which had proved satisfactory in previous studies, and consisted of 300 g. granulated sugar, 112 g. hydrogenated fat, 96 g. mixed whole egg, 6 g. salt, 284 g. cake flour, 8.4 g. double-acting baking powder, and 244 g. of whole milk. One-half of the basic formula was used for each baking. The amount of sugar or salt in the formula was not reduced by an amount equal to that added to the egg samples before they were frozen because it was felt that the home baker would not likely adjust her recipes to take account of the small amount of these ingredients present in the frozen egg. The frozen egg samples were thawed and brought to a temperature of 77°F.(25°C.) just before being used. The cakes were mixed by a uniform procedure by means of a household-size electric mixing machine. The batter for each mix was baked in two small loaf pans. Thus 210 g. of batter were weighed into each pan.

TABLE 1

Average volumes, water absorption values, and judges' scores on flavor and eating quality of plain cakes made from fresh eggs and from eggs frozen and stored at about 0°F.(—18°C.)

Period of storage of frozen eggs	Fresh shell eggs	Nothing added	Treatments of frozen eggs						Average for frozen egg cakes
			Salt		Sugar		Sirup		
			Teaspoons		Tablespoons		Tablespoons		
			1	2	1	2	1	2	
Average volumes[f] of cakes, ml.									
1 week	624	631	635	620	623	628	634	630	629
2 months	642	640	611	613	618	631	628	626	624
4 months	592	602	615	614	602	599	601	594	604
6 months	608	621	630	618	623	622	621	623	623
Average for all storage periods	617	624	623	616	617	620	621	618	
Average water absorption values, g.									
1 week	12.8	13.3	13.8	13.5	13.7	13.7	13.7	13.3	13.6
2 months	13.7	13.4	14.1	13.6	13.9	13.8	14.3	14.7	14.0
4 months	13.9	14.3	14.4	14.4	14.2	14.5	14.1	14.3	14.3
6 months	13.5	13.6	13.5	14.0	13.7	13.5	13.9	13.4	13.7
Average for all storage periods	13.5	13.6	14.0	13.9	13.9	13.9	14.0	13.9	
Judges' scores[g] on flavor									
1 week	12.5	12.7	12.7	12.2	13.1	13.3	13.4	13.1	12.9
2 months	13.0	13.1	12.3	11.3	13.6	13.3	13.3	13.5	12.9
4 months	13.6	13.2	12.7	11.8	13.5	13.1	13.1	13.5	13.0
6 months	13.0	12.6	12.5	12.5	13.1	13.1	13.1	13.0	12.8
Average for all storage periods	13.0	12.9	12.6	12.0	13.3	13.2	13.2	13.3	
Judges' scores[g] on eating quality									
1 week	11.8	12.7	12.6	12.5	13.1	13.1	13.2	12.9	12.9
2 months	12.9	12.7	12.6	11.9	13.4	13.2	13.3	13.5	12.9
4 months	13.0	13.1	12.7	11.9	12.9	12.9	12.7	13.1	12.8
6 months	12.8	12.6	12.7	12.2	12.7	13.1	13.2	12.9	12.8
Average for all storage periods	12.6	12.8	12.7	12.1	13.0	13.1	13.1	13.1	

[f] The volumes are from 210 g. of unbaked cake batter.
[g] Highest score possible, 15 points.

The observations and measurements made included the weight of the baked cake; observations on its external appearance; volume measured by seed displacement; the water absorbing ability of the cake (12); and quality as judged by the scores given by a panel of 5 judges on grain, quality of crumb, tenderness, velvetiness, flavor, and general eating quality.

A slice of cake from each baking was placed in a cellophane bag, sealed, and put into the freezer. At the end of the 6 months, samples from the 96 bakings were available for comparison as to size and grain.

Custards. The formulas for the baked custards were based upon the commonly used proportions of 1 whole egg (48 g.) or 2 yolks (36 g.) and 2 tablespoons of sugar (25 g.) to 1 cup (244 g.) of milk. One and one-half times the basic formula was used for the custard mixes. No reduction in the sugar was made to compensate for that present in treated frozen egg samples.

The frozen eggs or yolks were thawed just before they were combined with the sugar and the heated milk. Measured amounts of the custard mixture were poured into two glass custard cups. The duplicate cups were placed in a pan of hot water and the custards baked to an internal temperature of 190.4°F.(88°C.) in an oven heated to 350°F.(176.6°C.).

The baked custards were cooled, then placed in a refrigerator (temperature about 40°-45°F.) for one-half hour before they were tested for firmness by use of the Precision penetrometer and judged for firmness and palatability by a panel of three judges. The crusts were carefully removed from the custards before the cone of the penetrometer was released for a period of 5 seconds *(4)*.

RESULTS AND DISCUSSION

The average volumes of the baked cakes, the results of the water absorption test, and the judges' scores on the flavor and eating quality of the cakes are given in Table 1. An analysis of the data showed that there were no significant differences in the average volumes owing to the treatments given the eggs used. There was, however, a significant difference in the average volumes for the four storage periods. The average volumes of all the cakes with the exception of those made from the salt-treated eggs, were considerably lower at the 4-month storage period than for the other periods. No satisfactory explanation for this phenomenon is at hand.

Neither the treatments of the eggs nor the length of the storage periods affected the average water absorption values of the cakes baked from the frozen eggs.

The judges' scores for flavor and eating quality of the cakes made from the eggs treated with the higher level of salt were significantly lower than those for the other cakes. These lower scores were attributed to the excessively salty taste of these cakes. The cakes from the sugar and sirup-treated eggs scored somewhat higher in flavor than the fresh egg cakes, and those made from the untreated frozen eggs were almost equal to those made with fresh eggs. The storage periods did not significantly affect the flavor or eating quality scores. The cakes did not differ significantly in respect to grain, tenderness, or velvetiness.

The averages of the penetrometer readings and the judges' ranks for the firmness and flavor of the baked custards are given in Table 2. Both by the objective and subjective tests, the custards made from the fresh eggs were judged most firm. The differences in the average penetrometer readings for the whole-egg custards made from the 7 frozen egg samples were not statistically significant. Therefore, it is not surprising that the ranking in firmness given by the judges did not altogether agree with the

ranking shown by the penetrometer readings. On the other hand, the penetrometer readings for the egg-yolk custards did differ significantly, and in this case the rank in firmness given by the judges did agree with that shown by the objective test.

For both the whole-egg and the egg-yolk custards, the average penetrometer readings were significantly higher for the custards made from the frozen eggs stored for 4 months than for any of the other storage periods; even though significant, it may not be of too much practical importance.

The custards from the untreated frozen yolks were unsatisfactory; they were so soft that they were near liquid. The crust was thick and there were hard yellow lumps in it.

TABLE 2

Average penetrometer readings[b] (unit 0.1 mm.) and judges' ranking in firmness and flavor of custards made from fresh eggs and from eggs or egg-yolks frozen and stored at about 0°F.(—18°C.)

Period of storage of frozen eggs	Fresh shell eggs	Nothing added	Treatments of frozen eggs						Average for frozen egg custards
			Salt		Sugar		Sirup		
			Teaspoons		Tablespoons		Tablespoons		
			1	2	1	2	1	2	
Whole-egg custards									
1 week	281	278	278	265	281	274	273	272	274
2 months	255	275	271	260	271	266	273	271	270
4 months	266	277	286	274	276	282	286	280	280
6 months	267	272	273	269	270	270	269	268	270
Average for all storage periods	266	276	277	267	275	273	275	273	.....
Rank in firmness by penetrometer	1	7	8	2	5-6	3-4	5-6	3-4	.....
Rank in firmness by judges	1	6	8	5	2	4	3	7	.....
Rank in flavor by judges	5	6	7	8	4	3	1-2	1-2	.....
Egg-yolk custards									
1 week	295	339	296	287	301	289	314	293	297[1]
2 months	281	326	289	293	323	285	305	295	298[1]
4 months	285	.....	293	307	315	300	318	298	305[1]
6 months	277	.....	298	312	307	292	303	288	300[1]
Average for all storage periods	285	.....	294	300	312	291	310	294	.....
Rank in firmness by penetrometer	1	8	3-4	5	7	2	6	3-4	.....
Rank in firmness by judges	1	8	4	5	7	2	6	3	.....
Rank in flavor by judges	1	6	7	8	5	2	4	3	.....

[b] The lower the penetrometer reading the firmer the custard.

[1] These averages do not include the readings for the egg-yolk custards made from the "nothing added" samples.

The custards from the salt-treated eggs and egg yolks were undesirably salty in flavor. It should be noted that the levels of salt used for treating eggs and yolks fell within the amounts recommended in published directions for preparing eggs for home freezing *(2, 7, 14)*.

The untreated frozen whole eggs appeared somewhat curdled as they thawed, but they combined well with other ingredients and produced cakes and custards in many respects equal to those from fresh eggs. The untreated frozen yolks, however, were thick and gummy and did not combine well with the milk and sugar of custards. The salt-treated yolks, likewise, were very viscous and required considerable mixing to combine them with the other ingredients. The salt-treated whole eggs were of thinner consisteney than either the untreated or the sugar- or sirup-treated eggs.

There was no organoleptie evidence of any spoilage in any of the packages of frozen eggs. At the end of 6 months there were indications of slight dehydration in some of the cartons of untreated eggs and yolks.

SUMMARY

Egg yolks and whole egg magma, untreated and treated with 2 levels each of salt, sugar, and white corn sirup were frozen and stored in a home-type freezer at a temperature of about 0°F.(−18°C.). Samples were withdrawn from storage at the end of 1 week and after 2, 4, and 6 months for testing their culinary value in plain cakes and baked custards.

None of the pretreatments of the whole eggs affected significantly the firmness of custards, as measured by the penetrometer, or the volumes of the plain cakes.

The flavor of cakes and custards made from the sugar- or sirup-treated eggs was judged somewhat better than that of similar products made from the untreated and the salt-treated eggs. Products made from eggs treated with the higher level of salt were too salty to be desirable in flavor.

Under the conditions of this study, frozen eggs and yolks, with the exception of untreated yolks, retained to a high degree the functional properties necessary for satisfactory performance in plain cakes and custards.

LITERATURE CITED

1. Davis, E., Muir, J., and Sperry, T. A. Freezing farm products. State College of Washington Extension, *Bull.*, **230** (1947).
2. Diehl, H. C., and Warner, K. F. Freezing to preserve home-grown foods. U. S. Dept. Agr., *Circ.*, **709** (1945).
3. Haugh, R. R. The Haugh unit for measuring egg quality. *U. S. Egg Poultry Mag.*, **43**, 552 (1937).
4. Jordan, R., and Sisson, M. S. Use of spray-dried whole eggs in baked custards. *U. S. Egg Poultry Mag.*, **49**, 266 (1943).
5. LeClerc, J. A., and Bailey, L. H. Fresh, frozen, and dried eggs and egg products. *Cereal Chem.*, **17**, 279 (1940).
6. Lowe, Belle. *Experimental Cookery*. 3rd Ed., 1943, New York, N. Y.
7. Masterman, N. K., and Lee, F. A. The home freezing of farm products. N. Y. State College of Home Econ., Cornell Univ., *Bull.*, **611** (1943).
8. Miller, C., and Winter, A. R. The functional properties and bacterial content of pasteurized and frozen whole eggs. *Poultry Sci.*, **29**, 88 (1950).
9. Pearce, J. A., and Lavers, C. G. Liquid and frozen egg. V. Viscosity, baking quality, and other measurements on frozen egg products. *Can. J. Research*, **27F**, 231 (1949).
10. Schaible, P. J., and Card, C. G. How to produce frozen eggs that the housewife can use. *Food Inds.*, **15** (5), 67 (1943).

11. STEWART, G. F. Freeze up cheap spring eggs. *Farm Science Reporter*, **1** (2), 7 (1940).

12. SWARTZ, VENONA W. Two further simple objective tests for judging cake quality. *Cereal Chem.*, **15**, 247 (1938).

13. THOMAS, W., AND BAILEY, M. I. Gelation of frozen egg magma. *Ind. Eng. Chem.*, **25**, 669 (1933).

14. TRESSLER, D. K., AND EVERS, C. F. *The Freezing Preservation of Foods.* 1943. The Avi Publishing Co., New York, N. Y

15. URBAIN, O. M., AND MILLER, J. N. Relative merits of sucrose, dextrose, and levulose as used in the preservation of eggs by freezing. *Ind. Eng. Chem.*, **22**, 355 (1930).

16. United States Department of Agriculture. Egg and egg products. U. S. Dept. Agr., Circ. **583** (1941).

17. VANWAGENEN, A., AND WILGUS, JR., H. S. The determination and importance of the condition of the firm albumen in studies of egg-white quality. *J. Agr. Research*, **51**, 1129 (1935).

18. WINTER, A. R., AND WRINKLE, C. Frozen egg quality depends on clean eggs, clean equipment, and quick cooling. *U. S. Egg Poultry Mag.*, **55**, 7 (1949).

19. WOODROOF, J. G. Frozen eggs. *Ice and Refrig.*, **102**, 62 (1942).

20. WRINKLE, C., WEISER, H. H., AND WINTER, A. R., Bacterial flora of frozen egg products. *Food Research*, **15**, 91 (1950).

FATTY ACIDS OF THE LIPIDS FROM FRESHLY CANNED FLORIDA VALENCIA ORANGE JUICE[a]

LYLE JAMES SWIFT
U. S. Citrus Products Station,[b] Winter Haven, Florida

(Manuscript received April 5, 1951)

In another paper *(15)* the proximate analytical data on the lipids obtained from freshly canned Florida Valencia orange juice have been reported. Nolte and von Loesecke *(12)* have presented some analytical data on Florida Valencia orange juice lipids and Matlack *(11)* has reported on the lipids in California Valencia orange pulp. Nolte and von Loesecke *(12)* showed that rancidity changes occurred in the lipid of orange juice on storage. Curl *(2)* and Curl and Veldhuis *(3)* indicated that the off-flavors developing in tangerine and orange juices were associated with the lipids. From the conclusions of these authors, it seemed likely that the off-flavors might be due to the products resulting from the oxidation of unsaturated fatty acids, or that short-chain fatty acids might be liberated by hydrolysis of the lipid material during juice storage. The work reported herein was undertaken in order to learn what acids were present in the lipid material. The present paper reports analyses made to determine the amounts and the identity of the fatty acids of the lipids previously investigated by Swift and Veldhuis *(15)*.

EXPERIMENTAL METHODS

Lipid recovery. Orange juice in the previous work *(15)* had been prepared, briefly, as follows: 116 boxes of oranges (41 kg. per box) were hand-reamed and the juice was passed through a commercial finisher, deaerated, pasteurized at 95°C.(203°F.), and canned. Half of the cans were stored at laboratory loft temperatures, to simulate warehouse conditions, and the rest were kept at 0° to 5°C.(32° to 41°F.) to prevent or at least retard further changes. The lipids for the work reported in this paper were obtained from the juice that had been stored at 0° to 5°C. The juice was filtered in batches through filter-aid in Büchner funnels. The filter aid and its retained material were then extracted with acetone in a Soxhlet-type apparatus, the acetone was driven off from the extract by distillation at atmospheric pressure, and the residue was taken up in petroleum ether. The individual extracts were combined and evaporated. The lipids obtained amounted to 1,000 g. This represents a yield of 0.09% on the basis of the juice or 0.04% when calculated on the basis of the original fruit.

The term lipid, as used in this paper, includes everything extracted by acetone that is also soluble in petroleum ether. The proximate analysis of this fraction is given by Swift and Veldhuis *(15)*.

Separation of acids. First, 600 g. of the lipid was saponified by refluxing it for 2 hours with 500 g. of potassium hydroxide, 2.5 l. of ethanol, and 500 ml. of water. After distilling off most of the ethanol, the saponification mixture was diluted with water and the unsaponifiable matter was extracted with ethyl ether. The aqueous fraction was then acidified and the fatty acids were extracted with ethyl ether. Evaporation of an aliquot in a tared flask showed that 417 g. of acids had been obtained. A large amount of a deep-red substance which accompanied the acids was precipitated by distilling off

[a] Report of a study made under the Research and Marketing Act of 1946.

[b] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

the ethyl ether, followed by the addition of petroleum ether and filtration. However, the petroleum ether solution still retained considerable color. Part of the petroleum ether solution was dried with anhydrous sodium sulfate and filtered. The petroleum ether was then distilled off and 188.5 g. of acids were obtained. These acids of course include those that occur free in the lipid as well as those liberated by the saponification.

Preparation of esters. Esters of the 188.5 g. of acids were prepared in the usual manner by refluxing the acids with 750 ml. of absolute methanol and 7 ml. of concentrated sulfuric acid, removing the methanol by distillation under vacuum, taking up the esters in ethyl ether, and washing first, with sodium carbonate solution, and finally with water. Almost no acids remained in the washings, showing that the esterification was practically complete. The esters were obtained by distilling off most of the ethyl ether at atmospheric pressure and the rest under vacuum. The esters still retained the deep red color; thus the red substance had probably also been esterified since it was not removed by the carbonate washing. Colorless esters, 163.2 g., were obtained by a preliminary distillation of the esters at a pressure of 3-4 mm. of mercury in a Claisen flask.

Part of the esters were studied by spectrophotometric methods and part (118.9 g.) were fractionated on a column *(14)* at 1-2 mm. of mercury pressure. Seven fractions were collected. The flask residue and holdup (2.45 g.) were discarded because of extensive polymerization. The fractionation data are given in Table 1.

TABLE 1

Analytical data on ester fractions

Sample	Distilling temperature	Weight	Iodine value	Thiocyanogen value	Saponification equivalent	Saturated acids	Neutral equivalent of saturated acids	Saturated esters calculated
Mixed methyl esters	°C.	g.	107.33	64.44	288.22	% 20.73	262.65	% 21.83
Fraction 1	108-121	15.90	29.75	24.09	264.41	59.89	255.39	63.17
Fraction 2	121-131	16.90	37.59	26.38	273.04	61.30	259.17	64.61
Fraction 3	131-141	19.65	126.98	75.10	289.92	11.65	267.03	12.26
Fraction 4	141-142	21.15	145.61	85.27	294.63	1.43	330.46	1.49
Fraction 5	142	13.00	144.16	87.68	295.73	1.44	345.18	1.50
Fraction 6	142	21.10	139.76	88.30	297.74	2.34	330.65	2.44
Fraction 7	142	8.75	128.33	85.34	302.16	6.54	328.58	6.82

Analytical data. The iodine numbers on the mixed esters and the fractionated esters were determined by the Wijs method (30 minutes) and the thiocyanogen numbers were determined by the method of Lambou and Dollear *(9, 10)*. Saponification equivalents were determined by the method described by Jamieson *(7)*. Saturated acids were determined by the modified Bertram oxidation method. Neutral equivalents were obtained by titrating the saturated fatty acids in neutral ethanol in the usual manner. The percentages of saturated esters were calculated from the neutral equivalents and from the percentages of saturated acids and the corresponding molecular weights of their methyl esters. The data are given in Table 1.

Spectrophotometric data on the mixed esters. Spectrophotometric analyses, using the method of Brice, Swain, Schaeffer, and Ault *(1)*, were made on the mixed esters since satisfactory values for the unsaturated esters could not be calculated from the iodine and the thiocyanogen numbers and the percentage of saturated acids. The results of these analyses are given in Table 2. The results obtained by different investigators on the same sample of mixed esters are included for comparison.

RESULTS

Analyses of the ester fractions. Table 3 shows the percentages of saturated and unsaturated esters, on an unfractionated basis, of the seven fractions obtained on the fractionating column.

The unsaturated ester in Fraction 1 was palmitoleic. The exclusion of other unsaturated esters was established by subjecting a portion of the fraction to the lead salt-ether separation and oxidizing the separated portion by the method of Sullivan and Bailey *(13)*. The only oxidation product was 9,10-dihydroxy palmitic acid melting at 127-128°C. which is evidence for the presence of methyl palmitoleate. Since methyl palmitoleate was the only unsaturated ester found in Fraction 1, the amount present was calculated from the iodine value at 31.4%. Some saturated acids, recovered in purifying the above derivative, were found to be a mixture of 79% palmitic and 21% myristic acids, since the melting point was 58°C. and the neutral equivalent was 250, according to data given by Heintz *(5)*. The total proportion of saturated esters was 63.2% by the Bertram procedure. Thus about 5.4% of the saturated acids of Fraction 1 escaped the Bertram determination. It was assumed that this percentage had about the same composition as the saturated acids which appeared among the unsaturated acids resulting from the lead salt-ether separation.

TABLE 2

Spectrophotometric analysis of unfractionated esters

Substance	Analysis by	
	SRRL[d]	Author
Iodine number	107.0	107.3
Conjugated diene esters, %	1.31	1.27
Conjugated triene esters, %	0.189	0.156
Conjugated tetraene esters, %	0.048	0.163
Methyl linolenate, %	8.4	9.1
Methyl linoleate, %	33.1	31.8
Methyl oleate, %[c]	29.2	29.9
Saturated esters, % (by difference)	27.5	27.6

[c] This includes methyl palmitoleate. See Table 3 and text.
[d] The Southern Regional Research Laboratory.

The identity and percentage of palmitic and stearic esters were established in Fraction 3 from the neutral equivalent and solidification point of the saturated acids and the assumption was made that these same two esters were the constituents of the saturated part of Fraction 2, the proportion being calculated from the neutral equivalent of the Bertram acids. In Fractions 2 and 3 the molecular weights of the unsaturated esters were calculated from the saponification equivalents and the neutral equivalents of the saturated acids. From these molecular weights the proportions of C_{16} and C_{18} unsaturated esters were computed, using a value of 294. This is the midpoint between the molecular weights of methyl oleate and linolenate, respectively, the extremes for C_{18} unsaturated esters that could have been present.

The molecular weights of the saturated acids of Fractions 4 and 7 were established in a different manner since these acids have neutral equivalents too high to have distilled as esters with melocular weights under 302. Accordingly, a silver salt was made from part of the saturated acids of Fraction 7. From 0.1059 g. of this salt there was obtained upon ignition 0.0260 g. of silver which gave a molecular weight of 332 and confirmed the titration figures within the limits of experimental error.

Specific rotations were determined on a portion of the saturated fatty acids of the seven fractions. The specific rotations of Fractions 1, 2, and 3 were found to be zero; of Fractions 4, 5, and 6 totaled + 8.7°, and that of Fraction 7 was + 16.3°.

A portion of the saturated acids of Fractions 4 to 7, inclusive, (0.1400 g.) was acetylated and purified by conventional methods, giving 0.1446 g. of the acetylated product. This was dissolved in neutral ethanol and titrated to neutralize the free carboxyl groups, after which 30 ml. of 0.1012 *N*. sodium hydroxide was added and the mixture refluxed for 3 hours. A blank containing the same amount of neutral ethanol and alkali was refluxed for the same length of time. Back titration of samples and of the blank showed that 0.159 milliequivalents of base had been required to react with the acetyl group—that is, the acetyl radical amounted to 4.73% of the acetylated acids. These results, together with the optical activity and the irreconcilability between the high molecular weights of the saturated acids and relatively low molecular weights of the ester fractions, tend to support the presence of hydroxy acids produced from unsaturated acids by the permanganate oxidation. The average molecular weight, 344, of the saturated esters of the ester Fractions 4, 5, 6, and 7 (Table 3) is given, but it should be understood that these acids represent undoubtedly a mixture and probably contain some methyl stearate. The amount involved is small, however, so the error from this source is not great.

TABLE 3

Saturated and palmitoleic esters. Percent by fractions

	Fraction							Total
	1	2	3	4	5	6	7	
Methyl myristate, %	0.47	0	0	0	0	0	0	0.47
Methyl palmitate, %	9.06	8.47	1.29	0	0	0	0	18.82
Methyl stearate, %	0	0.92	0.78	0	0	0	0	1.70
Methyl ester,* mol. wt. 344, %	0	0	0	0.27	0.16	0.44	0.51	1.38
Methyl palmitoleate, %	4.31	4.25	1.82	0	0	0	0	10.38

*Probably contains methyl stearate. Based on saturated acids (Bertram) that contained hydroxy acids.

The saponification equivalents of Fractions 4, 5, 6, and 7 showed that only esters of C_{18} acids were present in the unsaturated fatty acids. It was still desirable to demonstrate the presence or absence of linolenate, methyl linoleate, and methyl oleate; the palmitoleate having been already confirmed in Fraction 1.

The presence of linolenic ester was confirmed as follows: First, 2.4 g. of the unsaturated acids, separated by the lead salt-ether process from Fraction 2, were brominated in a 10% dry ethyl ether solution by gradually adding bromine at a temperature of —17°C. The mixture was allowed to stand overnight at 5°C., and the precipitate was then filtered in a tared sintered glass crucible, washed with cold dry ether, and weighed. The 0.0657 g. obtained was recrystallized from xylene and was found to melt at 181-182°C. which agrees with the literature for stearic hexabromide, confirming the presence of linolenic ester.

The presence of methyl linoleate was shown as follows: The ether filtrate was freed of excess bromine by shaking with sodium thiosulfate solution, washed, evaporated, and the residue was taken up in petroleum ether and concentrated. After standing for about 2 days in the coldroom, a precipitate was obtained and separated by filtration. After three recrystallizations from petroleum ether the crystals melted at 113-114°C. which agrees with the melting point observed by other workers for tetrabromostearic acid. This is evidence for the presence of methyl linoleate in the ester mixture.

The presence of methyl oleate was shown as follows: The unsaturated fatty acids of Fraction 3 which had been separated by the lead salt-ether method in ethyl ether were brominated and the insoluble hexabromides were filtered off. The excess bromine was removed with amylene, followed by evaporating and digesting with petroleum ether. After allowing to stand in the coldroom, the insoluble precipitate was filtered off and the petroleum ether was evaporated from the filtrate. The residue was taken up in ethyl ether and debrominated by the method of Frankel and Brown *(4)* by refluxing with metallic zinc for 4 hours. The unused zinc and zinc bromide were filtered off and the ether was removed by distillation. The remaining acids were then oxidized with potassium permanganate by the method of Sullivan and Bailey *(10)*. By recrystallization from ethanol 9,10-dihydroxy stearic acid, melting at 133°C., was isolated. This is evidence for the presence of methyl oleate in the ester mixture.

The red substance. Although definitely not itself a fatty acid, it is probably not irrelevant to mention the red substance that accompanied the fatty acids. This is apparently responsible for much of the color of the lipids and of the juice, has definite acidic properties, and is not a carotene or carotenol. It probably possesses at least one carboxyl group since it was esterified and thereby lost its acidic properties. The fact that in the preliminary distillation of the esters the esterified red substance was left behind indicates that it had a high molecular weight or underwent some profound change such as polymerization. Its composition was not further investigated at this time.

Evaluation of the data on the fractionated esters

The justification for the fractional distillation of the esters lies in the aid it gave in detecting and estimating the palmitoleic ester and the saturated esters. Even with this help, however, the data leave much to be desired, especially in the first three fractions. For example, in Fraction 1 it was shown that myristic ester was present yet the neutral equivalent determination on the Bertram saturated acids gives no proof of this compound. Possibly it was not found by the Bertram determination because of the solubility of its magnesium salts in water, a point noted by Hilditch and Priestman *(6)*. Jamieson *(8)* also noted that myristic acid is not satisfactorily determined in either the lead salt-ether or lead salt-alcohol method because its lead salt remains wholly or partially with those of the unsaturated acids. This explains the appearance of this acid among the oxidation products of the unsaturated acids.

In Table 3 it will be noted that the saturated esters of the fractions totaled 22.37% and in Table 1 that those of the unfractionated mixture

amounted to 21.83%. While these figures are in good agreement, both values are much less than the 27.6% arrived at from the spectrophotometric data (Table 2). Moreover, part of the oleic ester determined from spectrophotometric data is palmitoleic ester, since the spectrophotometric method makes no distinction between these two esters. This fact has the effect of lessening the unsaturated esters and increasing the saturated esters since the latter is determined by difference.

SUMMARY

An analysis of the methyl esters of the fatty acids of freshly-canned Florida Valencia orange juice has been completed. The spectrophotometric method was used for the overall examination, and distilled fractions were applied for the saturated and palmitoleic esters. A considerable discrepancy was found in the percentages of saturated esters by the two methods. In Table 4 an overall summary of the ester composition is given, here the percentage of methyl oleate has been corrected for the palmitoleate content. All percentages have been expressed to the nearest tenth. The values for the unsaturated esters are based on averages of values given in Table 2.

TABLE 4

Summary of the total methyl ester composition

Constituent	Percent	Method of identification
Conjugated diene	1.3	Spectrophotometric
Conjugated triene	0.2	Spectrophotometric
Conjugated tetraene	0.1	Spectrophotometric
Linolenate	8.8	m.p. of hexabromide derivative
Linoleate	32.5	m.p. of tetrabromide derivative
Oleate (corrected)	18.1	m.p. of 9,10-dihydroxy derivative
Palmitoleate	10.4	m.p. of 9,10-dihydroxy derivative
Myristate	0.5	m.p. and neutral equivalent of saturated acids
Palmitate	18.8	m.p. and neutral equivalent of saturated acids
Stearate	1.7	m.p. and neutral equivalent of saturated acids
Unidentified, mol. wt. 344	1.4	.....
Not determined	6.2	.....
Total	100.0	

From the unsaturated acids found it is evident that there is ample opportunity for oxidative rancidity changes to take place and it is possible that these are the origin of the flavor deterioration. Since there appears to be no short-chain fatty acids present, it is not likely that hydrolysis of the lipid plays any major part in the flavor changes as far as the acids are concerned.

A red, acidic pigment was found in the course of this work. It appears to be of high molecular weight and capable of forming esters.

Acknowledgment

The author wishes to acknowledge his indebtedness to F. G. Dollear and J. R. Loeb of the Oil and Oilseed Division and to R. T. O'Connor and Elsie T. Field of the Analytical and Physical Division, all of the Southern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, for the spectrophotometric analytical

data presented in Table 2. Also, many thanks are due to B. A. Price and A. Turner of the Analytical and Physical Division and to R. W. Riemenschneider and S. F. Herb of the Oil and Fats Division, all of the Eastern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, for their advice and suggestions that made the author's spectrophotometric data possible.

LITERATURE CITED

1. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C. Spectrophotometric determination of small portions of polyunsaturated constituents in fatty materials. *Oil and Soap*, **22**, 219 (1945).
2. Curl, A. L. Off-flavor development in processed tangerine juice. *Fruit Products J.*, **25**, 356 (1946).
3. Curl, A. L., and Veldhuis, M. K. The origin of the off flavor which develops in processed orange juice. *Fruit Products J.*, **26**, 329 (1947).
4. Frankel, J. S., and Brown, J. B. Chemistry of the fatty acids. XII. The preparation of the alpha- and beta-linoleic acids by debromination in various solvents and observations on the chemistry of these acids. *J. Am. Chem. Soc.*, **65**, 415 (1943).
5. Heintz. Liebig's Ann., **92**, 295. Cited in Lewkowitsch, J., *Chemical Technology and Analysis of Oils, Fats, and Waxes*. 1921, Vol. I. Macmillan and Co., London, England.
6. Hilditch, T. P., and Priestman, J. Rapid determination of solid saturated fatty acids. *Analyst*, **56**, 354 (1931).
7. Jamieson, G. S. *Vegetable Fats and Oils*. 1st Ed., 1932, Chemical Catalog Co., New York, N. Y.
8. Jamieson, G. S. *Vegetable Fats and Oils*. 2nd Ed., 1943, Reinhold Pub. Corp., New York, N. Y.
9. Lambou, M. G., and Dollear, F. G. Modified thiocyanogen reagent and method. *Oil and Soap*, **22**, 1 (1945).
10. Lambou, M. G., and Dollear, F. G. A preparation of lead thiocyanate. *Oil and Soap*, **23**, 1 (1946).
11. Matlack, M. B. The fatty constituents of California Valencia orange pulp. *J. Org. Chem.*, **5**, 504 (1940).
12. Nolte, A. J., and von Loesecke, H. W. Chemical and physical characteristics of the petroleum ether-soluble material of fresh and canned Florida Valencia orange juice. *Food Research* **5**, 457 (1940).
13. Sullivan, B., and Bailey, C. H. Lipids of the wheat embryo. I. *J. Am. Chem. Soc.*, **58**, 383 (1936).
14. Swift, L. J. Tangerine seed oil. *J. Am. Oil Chem. Soc.*, **26**, 438 (1949).
15. Swift, L. J., and Veldhuis, M. K. Constitution of the juice lipids of the Florida Valencia orange. *Food Research*, **16**, 142 (1951).

EFFECT OF BLANCHING AND OF FROZEN STORAGE OF VEGETABLES ON ASCORBIC ACID RETENTION AND ON THE CONCOMITANT ACTIVITY OF CERTAIN ENZYMES[a]

EVA R. HARTZLER[b] AND N. B. GUERRANT
The Pennsylvania State College, State College, Pennsylvania

(Manuscript received April 17, 1951)

The present studies were carried out in an effort to determine the relationship, if any, between the inactivation of certain enzymes and the retention of ascorbic acid and of other quality factors in frozen vegetables. It was desired to ascertain which of the enzymes are causative factors in the losses of ascorbic acid and in the deterioration of quality in frozen foods. Furthermore, it seemed desirable to determine what degree of enzyme inactivation is required in order to reduce or prevent these changes.

The literature contains extensive data relating to the effects of blanching and of frozen storage on the retention of ascorbic acid in vegetables *(14)*. In recent years there also have been published the results of a number of studies relative to the inactivation of enzymes, particularly catalase and peroxidase, and the retention of quality in frozen vegetables *(5)*. There have been very few reports, however, in which the effect of blanching on both the ascorbic acid content and on enzyme activity have been studied simultaneously.

In the present studies, the ascorbic acid content and the activities of catalase, ascorbic acid oxidase, peroxidase, and polyphenol oxidase in broccoli, green beans, Lima beans, peas, spinach, and in summer squash were determined. These determinations were made on samples of the vegetables before and after three degrees of blanching and before and after various periods of frozen storage at 0°F.(—17.8°C.). These six vegetables represented various types of plant tissues as leaves, flowers, immature seeds, etc.

EXPERIMENTAL METHODS

Processing of the vegetables. Fresh vegetables of good quality were first washed and where necessary cut into appropriate size. The heads of broccoli (DeCicco variety) were sub-divided, all large leaves being removed, and the stalks were cut into pieces 4½ inches or less in length. Stalks which were more than ½ inch thick were also cut lengthwise. The green beans (Bountiful variety) were first washed, the ends snipped off, and the beans broken into pieces 1 to 2 inches in length. The spinach (Bloomsdale variety) was sorted, washed twice in cold water, and allowed to drain for an hour before being blanched. Small Zucchini squash, 6 to 8 inches long and 1½ to 3 inches in diameter, were used. They were first washed and then cut into slices about ½ inch in thickness. Freezonian peas and Henderson's bush Lima beans were used.

The hand picked vegetables were blanched for ½ to 4 minutes, in water at 200°F. (93.3°C.) using a ratio of 1 pound of vegetable to 1 gallon of blanching water. After

[a]Authorized for publication on April 5, 1951, as paper No. 1665 in the Journal series of the Pennsylvania Agricultural Experiment Station.

[b]Present address: Department of Chemistry, Juniata College, Huntingdon, Pennsylvania.

blanching for the designated length of time, the vegetables were quickly drained and cooled in iced water. The chilled vegetables were then drained, reweighed, and packed into tin cans which were sealed and placed in a freezing unit which maintained a temperature of 0°F.(−17.8°C.).[6] The frozen vegetables were removed at designated intervals for ascorbic acid analysis and for enzyme tests.

Determination of moisture. Moisture determinations were made on the raw and on the blanched samples before freezing. On the day the vegetables were processed for freezing, duplicate 100-g. portions of both the unblanched and the blanched vegetables, together with 100-ml. portions of distilled water were sealed in tin cans, autoclaved and then set aside for the determination of the moisture content at some future date. Later the entire contents of the can were blended thoroughly in a Waring Blendor and the moisture content of the purée was determined in a Brabender Moisture Tester at 185°F.(85°C.).

Determination of ascorbic acid. The ascorbic acid content of all of the samples of vegetables except spinach was determined by the conventional dye-titration method. Inasmuch as spinach yielded highly colored extracts, the method of Loeffler and Ponting *(7)* as modified by Ponting *(10)* was used in determining the ascorbic acid content of this vegetable.

In all instances extracts were prepared by blending 40 g. of the vegetable with 200 ml. of 0.5% oxalic acid for 3 minutes in a Waring Blendor and filtering the purée through coarse filter paper. The filtrates were then assayed either by the direct dye-titration or by the colorimetric procedure.

Determination of enzyme activity. Extracts were prepared by blending duplicate 40-g. portions of the vegetables for 3 minutes with 120 ml. of chilled 0.2 *M*. phosphate-0.1 *M*. citrate buffer (pH 7.0 or 8.0) and filtering through a double layer of cheesecloth. Peas and green beans were extracted with a buffer of pH 7.0 and the remaining four vegetables with a buffer of pH 8.0. The choice of pH was made on the basis of preliminary tests in which water and phosphate buffers of pH 6.0, 7.0, and 8.0 were used. Low enzyme activities were always found when water was used and the activities with a buffer of pH 6.0 were always lower than with buffers of pH 7.0 or 8.0. The buffer which yielded vegetable extracts with the greatest activity was used throughout the experiment for that vegetable. The enzyme-containing filtrate was allowed to stand in a refrigerator for 1 to 2 hours and then was decanted from the residue. The cloudy, greenish decanted extracts were held in stoppered flasks until used.

Assays for the various enzymes were carried out on the above extracts after diluting to suitable concentrations. Dilution of extracts for each enzyme assay was made with the buffer used in the assay of that particular enzyme. Ascorbic acid oxidase activity was determined by two methods: the manometric method of Lovett-Janison and Nelson *(8)* and the colorimetric method of Ponting and Joslyn *(11)*. Catalase determinations were made by the manometric method described by Mallette, Dawson, Nelson, and Gortner *(9)*. Two methods were also used for measuring peroxidase activity: the colorimetric method described by Ponting and Joslyn *(5)*, and the ascorbic acid method described by the same authors. Polyphenol oxidase activity was determined by the manometric method of Adams and Nelson *(1)*. The manometric determinations were carried out at 85°F. (29.5°C.) during a period of 20 minutes. In the colorimetric methods, which were carried out at temperatures from 80.5° to 86°F.(27° to 30°C.), ascorbic acid oxidase was allowed to react for 3 minutes and peroxidase activity was measured in the time interval between the first and second minute after the addition of the enzyme. All values were calculated in terms of enzyme activity (expressed as μl. of O_2 absorbed or evolved, γ or mg. of ascorbic acid destroyed, or as changes in density expressed as "L" value) per gram of vegetable per minute.

RESULTS

Changes in weight and in moisture content during blanching. The changes in weight which occurred during blanching and the moisture contents of the vegetables before and after blanching are shown in Table 1. It will be noted that weight changes were relatively small for all vegetables

[6] Furnished through the courtesy of Sears, Roebuck and Company.

except spinach which lost 15 to 25% of its original weight during blanching. On the basis of these findings, the vitamin values for spinach were calculated to the fresh weight basis, whereas the values for the other vegetables are reported without making corrections for the small weight changes which occurred. The measured moisture values were used in calculating the equivalent concentration of the vegetable extracts used for assay purposes.

TABLE 1

Changes in weight occurring during blanching and the moisture contents of the six vegetables before and after blanching

Sample	Blanching time	Weight			Moisture
		Before blanching	After blanching	Change	
	minutes	*g.*	*g.*	%	%
Broccoli	0	.....	.....	.....	89.6
Broccoli	1.0	2,000	2,134	+6.5	90.9
Broccoli	2.0	2,300	2,417	+5.0	91.5
Broccoli	4.0	2,300	2,440	+6.0	91.5
Green beans	0	.....	.....	.....	92.3
Green beans	0.75	2,300	2,305	0	92.3
Green beans	2.0	2,300	2,292	0	91.6
Green beans	4.0	2,300	2,252	−2.0	92.3
Lima beans	0	.....	.....	.....	72.4
Lima beans	0.75	.....	.....	.....	71.2
Lima beans	2.0	11,500	11,068	−3.8	72.4
Lima beans	4.0	11,500	11,336	−1.4	73.0
Peas	0	.....	.....	.....	81.2
Peas	0.5	2,250	2,110	−6.2	82.3
Peas	1.5	11,364	10,471	−7.9	81.2
Peas	3.0	11,364	10,622	−6.0	81.3
Spinach	0	.....	.....	.....	93.6
Spinach	0.5	2,400	2,030	−15.0	92.4
Spinach	1.5	2,400	1,940	−19.0	92.9
Spinach	3.0	2,300	1,738	−25.0	93.2
Squash	0	.....	.....	.....	95.0
Squash	0.75	2,500	2,654	+6.0	95.0
Squash	2.0	2,500	2,646	+6.0	95.3
Squash	4.0	2,500	2,488	−0.5	94.4

Loss of ascorbic acid. The effects of blanching, freezing, and frozen storage on the ascorbic acid content of the six vegetables are shown in Figures 1-6, inclusive. Blanching resulted in marked losses of ascorbic acid in every case. Broccoli and spinach showed a progressive decrease in ascorbic acid content with increasing blanching time. With peas and green beans, however, the greatest loss of ascorbic acid was observed in the samples receiving the shortest blanch while with squash the greatest loss occurred in the samples receiving the medium length blanch.

Additional losses of ascorbic acid occurred during freezing and during frozen storage. The losses during freezing were particularly marked in the unblanched samples of broccoli and spinach and in both blanched and

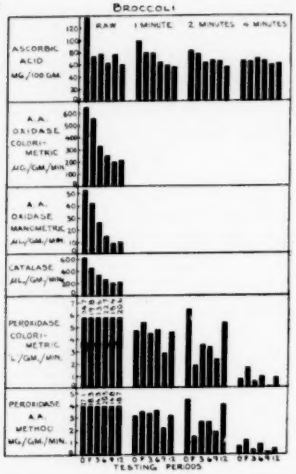


Figure 1. The effect of blanching, freezing, and frozen storage on the ascorbic acid content and on the enzyme activity of broccoli.

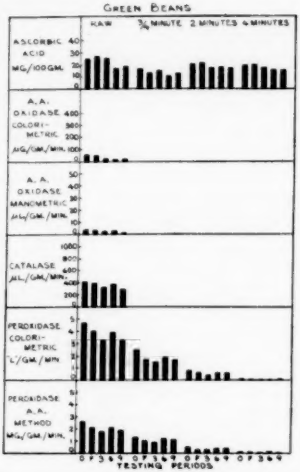


Figure 2. The effect of blanching, freezing, and frozen storage on the ascorbic acid content and on the enzyme activity of green beans.

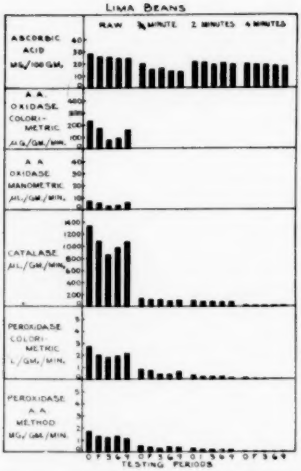


Figure 3. The effect of blanching, freezing, and frozen storage on the ascorbic acid content and on the enzyme activity of lima beans.

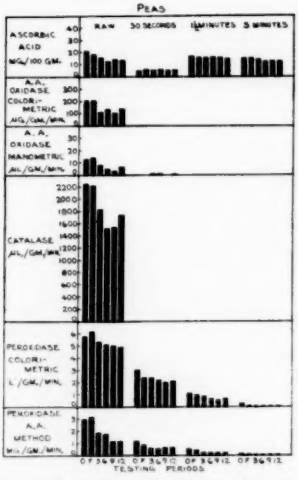


Figure 4. The effect of blanching, freezing, and frozen storage on the ascorbic acid content and on the enzyme activity of peas.

unblanched samples of squash. Practically all of the samples underwent some loss of ascorbic acid during storage for 9 months at 0°F.(—17.8°C.) and in most instances the greatest loss occurred in the unblanched samples. The stability of ascorbic acid in both broccoli and spinach showed a progressive increase with increased blanching time.

Enzyme activity. The values for the activities of the various enzymes (except polyphenol oxidase) are shown in Figures 1-6. The data for polyphenol oxidase are presented in Table 2. When fresh unblanched vegetables were tested, catalase and peroxidase were found in all samples investigated. Ascorbic acid oxidase was also found in all vegetables with the possible exception of spinach, where the activity was so slight as to be of doubtful significance. Laccase, for which qualitative tests were made, was not found in any of the six vegetables. Polyphenol oxidase was found to be present only in spinach and squash, and in these two vegetables the enzymic activity was low.

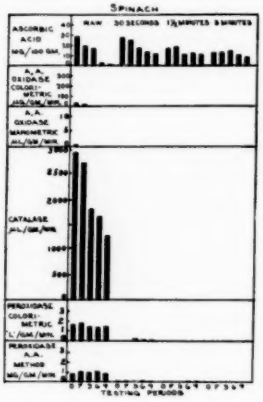


Figure 5. The effect of blanching, freezing and frozen storage on the ascorbic acid content and on the enzyme activity of spinach.

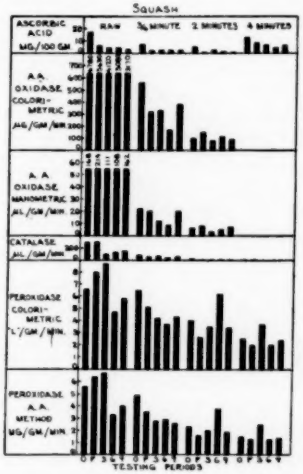


Figure 6. The effect of blanching, freezing, and frozen storage on the ascorbic acid content and on the enzyme activity of squash.

Blanching resulted in marked losses of activity of each of the enzymes studied and the extent of inactivation increased with increased blanching time. Ascorbic acid oxidase was the most easily inactivated and was apparently completely inactivated in all of the vegetables except squash by the shortest blanch used. Catalase also was quite easily inactivated although it was slightly more resistant to the heat treatment than was ascorbic acid oxidase. Peroxidase was considerably more resistant to the heat treatment than were the other enzymes studied. Significant amounts of this enzyme often remained after a medium length blanch and at least

traces of it remained after the longest blanch used with all vegetables except with spinach. Polyphenol oxidase also appeared to be very readily inactivated by blanching, but it was present in such small amounts that it may be illogical to compare it with the other enzymes concerned in this study.

These findings relative to the sensitivity of the various enzymes to heat inactivation agree well with those of other workers. Kertesz, Dearborn, and Mack *(6)* reported that ascorbic acid oxidase in peas is slightly more readily inactivated than is catalase. Bedford and Joslyn *(2)* found that ascorbic acid oxidase and catalase were inactivated at about the same rate in green beans and that both were more readily inactivated than peroxidase.

TABLE 2

Values obtained for polyphenol oxidase activity in unblanched spinach and squash

Test period	Activity, μl. O_2 per g. per minute	
	Spinach	Squash
Before freezing	3	3
After freezing	4	2
3 months	5	2
6 months	3	2
9 months	5	3

In most instances some loss of enzyme activity occurred as a result of freezing. Ascorbic acid oxidase and catalase activities decreased in almost all instances. Although values for peroxidase activity also were generally lower after freezing, higher values were obtained in some samples of broccoli and squash. These apparent increases, however, were probably due to sampling variability since both of these vegetables were relatively high in peroxidase and the individual pieces of the vegetables were relatively large. In order to reduce the error of sampling, the entire contents of each can were chopped and well mixed before sampling. With materials as variable in size as broccoli and squash, however, there is always the possibility of a relatively large sampling error.

Further losses of ascorbic acid oxidase and catalase activity occurred during frozen storage. The effect of storage on the peroxidase values was somewhat erratic, particularly in the instance of broccoli and squash, but in most cases there was also a loss in activity of this enzyme, although this loss was not nearly so marked as with the former two enzymes.

DISCUSSION

It is difficult to make generalizations or to draw conclusions from the data obtained in these studies because of the variation in the behavior of the different types of vegetable tissues studied. In some instances vegetables which were high in enzyme activity were relatively stable with respect to ascorbic acid and, in other instances, certain vegetables with relatively low enzyme activity were very unstable with respect to this substance. With some vegetables, however, there appeared to be a correlation between inactivation of the enzymes and retention of ascorbic

acid. Broccoli and spinach, for example, showed a progressive increase in stability of ascorbic acid during freezing and storage with increased blanching time. Likewise, with green beans, peas, and squash, the samples receiving the longest blanch were the most stable with respect to ascorbic acid.

With spinach, however, there seemed to be little or no correlation between enzyme activity and the retention of ascorbic acid. This vegetable, which was notable for its poor retention of ascorbic acid, was very low in ascorbic acid oxidase activity and was lower than any of the other five vegetables in peroxidase activity. Spinach was found to be high in catalase which might indicate that catalase was the responsible agent. However, in spite of the fact that the shortest blanch (30 seconds) reduced the catalase activity to zero and the peroxidase to near-the-zero levels, the samples subjected to this blanch were still relatively unstable with respect to ascorbic acid. This might mean that the large losses of ascorbic acid from spinach were due to non-enzymic catalysis as suggested by Kertesz, Dearborn, and Mack *(6)*. In that case, however, it is difficult to explain the progressive increase in stability which occurred with increased blanching time. The increase in stability of ascorbic acid which resulted from blanching for only 30 seconds seems to indicate that enzyme inactivation is an important factor.

That catalase is probably not responsible for the losses of ascorbic acid is indicated by the finding that Lima beans, which were quite stable with respect to ascorbic acid, were relatively high in catalase. Furthermore, catalase activity was very low in certain blanched samples of squash and broccoli, as well as in spinach, all of which were unstable with respect to ascorbic acid.

At first glance it seems probable that peroxidase may be responsible for the losses of ascorbic acid since it was present in each of the vegetables studied and was the enzyme most resistant to inactivation. Although high values for peroxidase frequently occurred in samples which were unstable with respect to ascorbic acid, it was also present in relatively large amounts in some vegetables, for example, green beans and Lima beans, which were relatively stable, with respect to this vitamin. In general, the quantitative relationship between peroxidase activity and the stability of ascorbic acid was not uniform enough to indicate a correlation between the two.

Although the question of which enzyme or enzymes are responsible for the changes which occur remains unanswered, the data presented provide support for the contention that inactivation of enzymes by blanching is an important factor in the retention of ascorbic acid. The data also show that not only over-blanching but also under-blanching may cause excessive losses of ascorbic acid. In fact the largest losses of ascorbic acid observed in this study occurred as a result of under-blanching.

Researchers have observed excessive losses of ascorbic acid following under-blanching in which low temperatures, rather than short blanching periods, were used. Todhunter and Sparling *(13)* observed that peas blanched at 160°F.(71°C.) lost much more ascorbic acid than did those blanched at 210°F.(99°C.), and suggested that perhaps the ascorbic acid oxidase was not completely inactivated at the lower temperature.

Guerrant et al. *(4)* reported that green beans blanched at 160°F.(71°C.) lost much more ascorbic acid than beans blanched at higher temperatures. Guerrant and Dutcher *(3)* reported that beans blanched at low temperatures not only lost more ascorbic acid during blanching but also lost large amounts during holding at room temperature, whereas, unblanched beans or beans blanched at 200°F.(93.3°C.) lost very little ascorbic acid during holding. These authors suggested that the observed effects were probably due to some enzyme system which was apparently activated by blanching at low temperatures.

Robinson, Moyer, and Kertesz *(12)* reported that peas blanched at 161.5°F.(72°C.) lost large amounts of ascorbic acid during blanching and continued to lose ascorbic acid during subsequent holding at room temperature, whereas, peas blanched at 176°F.(80°C.) or above lost no ascorbic acid during holding at room temperature for two hours. These authors suggested that perhaps the temperature used was sufficient to disrupt the tissues so that ascorbic acid could be destroyed by the ascorbic acid oxidase present and that the increased temperature during blanching accelerated the enzyme action.

Apparently the factors involved in the loss of ascorbic acid and in the deterioration of quality in frozen vegetables are quite complex inasmuch as each vegetable seems to present an individual problem. Although it is probably necessary to completely inactivate ascorbic acid oxidase in order to prevent excessive losses of ascorbic acid, the results obtained in the present studies with several vegetables indicate that merely to inactivate this enzyme is not always sufficient to insure ascorbic acid retention. In order to prevent excessive losses of this vitamin, it is also important, to employ a blanch severe enough to bring this inactivation to rapid completion.

SUMMARY AND CONCLUSIONS

A study has been made of the effects of blanching, freezing, and frozen storage on the retention of ascorbic acid and on the inactivation of certain enzymes in six different vegetables. Each of the vegetables (broccoli, green beans, Lima beans, peas, spinach, and squash) was blanched in water at 200°F.(93.3°C.) for three different periods so as to cause, insofar as possible, under-blanching, adequate-blanching, and over-blanching. Assays were made for ascorbic acid, ascorbic acid oxidase, catalase, peroxidase, and polyphenol oxidase on both blanched and unblanched samples before and after freezing and after storage for 3, 6, and 9 months at 0°F.(—17.8°C.).

Losses of ascorbic acid resulting from blanching were greatest with the shortest blanch for peas and green beans, with the medium blanch for squash, and with the longest blanch for broccoli and spinach. Losses of ascorbic acid during freezing and frozen storage were greatest in the unblanched and under-blanched vegetables.

Blanching greatly reduced the activity of the enzymes. Peroxidase was more resistant to the heat treatment than was ascorbic acid oxidase or catalase. Freezing and frozen storage resulted in further smaller decreases in enzyme activity.

The results of the present studies indicated no uniform relationship between enzyme activity and stability of ascorbic acid.

LITERATURE CITED

1. ADAMS, MARK H., AND NELSON, J. M. On the nature of the enzyme tyrosinase. *J. Am. Chem. Soc.*, **60**, 2474 (1938).
2. BEDFORD, C. L., AND JOSLYN, M. A. Enzyme activity in frozen vegetables—stringbeans. *Ind. Eng. Chem.*, **31**, 751 (1939).
3. GUERRANT, N. B., AND DUTCHER, R. ADAMS. Further observations concerning the relationship of temperature of blanching to ascorbic acid retention in green beans. *Arch. Biochem.*, **18**, 353 (1948).
4. GUERRANT, N. B., VAVICH, M. G., FARDIG, O. B., ELLENBERGER, H. A., STERN, R. M., AND COONEN, N. H. Nutritive value of canned foods. XXIII. Effect of duration and temperature of blanch on vitamin retention by certain vegetables. *Ind. Eng. Chem.*, **39**, 1000 (1947).
5. JOSLYN, M. A. Enzyme activity in frozen vegetable tissue. Advances in Enzymology **9**, 613 (1949).
6. KERTESZ, Z. I., DEARBORN, R. B., AND MACK, G. L. Vitamin C in vegetables. IV. Ascorbic acid oxidase. *J. Biol. Chem.*, **116**, 717 (1936).
7. LOEFFLER, H. J., AND PONTING, J. D. Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. *Ind. Eng. Chem., Anal. Ed.*, **14**, 846 (1942).
8. LOVETT JANISON, P. L., AND NELSON, J. M. Ascorbic acid oxidase from summer crook-neck squash (C. pepo condensa). *J. Am. Chem. Soc.*, **62**, 1409 (1940).
9. MALLETTE, M. F., DAWSON, C. R., NELSON, W. L., AND GORTNER, W. A. Commercially dehydrated vegetables. Oxidative enzymes, vitamin content, and other factors. *Ind. Eng. Chem.*, **38**, 437 (1946).
10. PONTING, J. D. Extraction of ascorbic acid from plant materials. Relative suitability of various acids. *Ind. Eng. Chem., Anal. Ed.*, **15**, 389 (1943).
11. PONTING, J. D., AND JOSLYN, M. A. Ascorbic acid oxidation and browning in apple tissue extracts. *Arch. Biochem.*, **19**, 47 (1948).
12. ROBINSON, W. B., MOYER, J. C., AND KERTESZ, Z. I. "Thermal maceration" of plant tissue. *Plant Physiol.*, **24**, 317 (1949).
13. TODHUNTER, E. N., AND SPARLING, B. L. Vitamin values of garden-type peas preserved by frozen-pack method. I. Ascorbic acid (vitamin C). *Food Research*, **3**, 489 (1938).
14. TUCKER, RUTH E. Acceptability and ascorbic acid content of frozen Rhode Island vegetables. Rhode Island Agr. Expt. Sta., *Bull.*, **302** (1948).

THE STABILITY OF THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID OF LAMB CHOPS DURING FROZEN STORAGE AND SUBSEQUENT COOKING[a]

W. P. LEHRER, JR., A. C. WIESE, W. R. HARVEY, AND P. R. MOORE[b]
Idaho Agricultural Experiment Station, Moscow, Idaho

(Manuscript received April 21, 1951)

Information regarding thiamine, riboflavin, and nicotinic acid content of raw and frozen lamb is limited. Kellogg *(3)* reported that lamb tissues contain approximately 1 to 3 μg. of thiamine per gram of tissue and in general more riboflavin than thiamine. Studies by Darby and Day *(2)* indicated that fresh lamb chops contained 2.8 μg. of riboflavin per gram of tissue. Waisman and Elvehjem *(8)* in their complete vitamin study of meats state the thiamine, riboflavin, and nicotinic acid content of fresh raw lamb leg is 2.6 to 3.3 μg., 2.8 to 3.6 μg. and 0.085 mg. per gram of tissue, respectively. Campbell, Hiltz, and Robinson *(1)* have found that the thiamine content of raw lamb chops is 0.34 to 1.18 μg. per gram sample and for cooked lamb chops 0.44 to 1.30 μg. per gram sample.

Frozen storage of meats has become one of the principal methods of meat preservation. In view of this fact, it was deemed advisable to study the effect of freezing, thawing, and cooking on the thiamine, riboflavin, and nicotinic acid content of various meats from animals of known nutritional history. The material presented in this paper covers studies on lamb chops.

EXPERIMENTAL PROCEDURE

Lamb chops used in these studies were obtained from carcasses processed in the Department of Animal Husbandry meat laboratory. The 6 choice carcasses were from ewe lambs of the Suffolk breed, bred and fed by the Experiment Station. The lambs used in these studies weighed 95-105 lbs. live-weight and were out of ewes fed comparable rations. The lambs were all fed the same ration throughout the growing and fattening period.

Twenty-four hours after slaughter the left back (racks and loins) was removed from the carcasses between the 5th and 6th ribs and cut into uniform size chops ¾ inch in thickness. Each chop was individually numbered from the rib end and processed according to the schedule presented in Table 1. The 16 packages of chops that were to be frozen were first wrapped in double-treated waxed freezer paper and then in regular heavy butcher paper. Freezer paper was placed between the chops prior to wrapping.

The 16 packages of lamb chops were spread out on shelves in the two freezers. Eight packages were frozen and stored at the generally recommended freezer temperature of 0°F.(−18°C.) and the other eight packages were frozen and stored at −15°F. (−26°C.). Both freezers were cooled with still air.

Where indicated in Table 1, the "thawed" (T) chops were thawed at room temperature, 70°F.(21°C.), and those "cooked" were either cooked in a frozen state (C.F.) or thawed at room temperature (T.C.) and then pan fried as recommended by the National Livestock and Meat Board *(4)*.

---

[a] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper 327.

[b] Present address: Department of Biochemistry, University of Louisville Medical School, Louisville, Kentucky.

In all vitamin determinations, the longissimus dorsi muscle was used for analysis. The muscle was cut out of the chop, fat trimmed off, and then finely ground.

Thiamine was determined by a modification of the method as described by Michelsen, Condiff, and Keyes *(5)*; riboflavin was measured microbiologically by the method of Snell and Strong *(6)* and nicotinic acid by the method of Snell and Wright *(7)*. Duplicate determinations were made on all samples. The results are reported on an oven-dry (95°C.) basis.

TABLE 1

Treatment of lamb chops

Package number	Chops[c]						Freezing treatment	Storage period	Treatment at time of analysis
	A	B	C	D	E	F			
I	1	17	14	11	8	5	Not frozen	None	Raw
II	2	18	15	12	9	6	−15°F.	24 hours	Thawed
III	3	1	16	13	10	7	0°F.	48 hours	Thawed
IV	4	2	17	14	11	8	−15°F.	3 months	Thawed
V	5	3	18	15	12	9	0°F.	3 months	Thawed
VI	6	4	1	16	13	10	−15°F.	3 months	Cooked—frozen
VII	7	5	2	17	14	11	0°F.	3 months	Cooked—frozen
VIII	8	6	3	18	15	12	−15°F.	3 months	Thawed—cooked
IX	9	7	4	1	16	13	0°F.	3 months	Thawed—cooked
X	10	8	5	2	17	14	−15°F.	6 months	Cooked—frozen
XI	11	9	6	3	18	15	0°F.	6 months	Cooked—frozen
XII	12	10	7	4	1	16	−15°F.	6 months	Thawed—cooked
XIII	13	11	8	5	2	17	0°F.	6 months	Thawed—cooked
XIV	14	12	9	6	3	18	−15°F.	6 months	Thawed
XV	15	13	10	7	4	1	0°F.	6 months	Thawed
XVI	16	14	11	8	5	2	−15°F.	48 hours	Thawed
XVII	17	15	12	9	6	3	0°F.	24 hours	Thawed
XVIII	18	16	13	10	7	4	Not frozen	None	Cooked

[c] Chop number refers to location from which the chop was cut beginning with the 6th rib and from the rib end of the back.

RESULTS AND DISCUSSION

The means of duplicate determinations for each of the six backs and for each of the 18 treatments are presented in Table 2. Preliminary analysis of these data indicated that the mean and variance are positively correlated. A correlation of this kind causes the pooled error term in the analysis of variance to be heterogenous and thus invalidates many of the tests of significance. In order to eliminate or greatly reduce the correlation between the mean and variance, the data were transformed to logarithms prior to the statistical analysis.

As will be noted from Table 1, chops from the same location of the back did not receive every treatment. Therefore, location differences, if they exist, are confounded with treatment differences. The amount of confounding is small, however, since chops from nearly the same location of the back did receive every treatment. Even so, accurate estimates of individual location effects cannot be made from these data.

On the other hand, an unbiased estimate of the component of variance for location can be made by separating the seventeen degrees of freedom for locations from the 85 (or 55) for back × treatment interaction. The expectations of the mean squares are then determined, the computed values are set equal to the expected values, and the resulting set of equations are solved. As an example of the use of this method of estimation, the mean

TABLE 2

Mean content of thiamine, riboflavin, and nicotinic acid (μg. per gram of dry tissue) in the longissimus dorsi muscle of lamb chops from six backs (loins and racks) [d]

	Fresh		Storage times																Back means
			24 hours Thawed and analyzed		48 hours Thawed and analyzed		3 months						6 months						
							−15°F.			0°F.			−15°F.			0°F.			
	Raw	Fried	−15°F.	0°F.	−15°F.	0°F.	T.	T.C.	C.F.	T.	T.C.	C.F.	T.	T.C.	C.F.	T.	T.C.	C.F.	
Back "A"																			
Thiamine	11.6	4.3	4.4	8.0	9.4	4.8	5.8	4.6	5.2	6.6	3.2	3.0	11.2	4.6	4.3	6.6	4.0	3.8	5.9
Riboflavin	....	....	....	....	....	....	8.6	8.6	5.4	9.8	6.7	9.7	7.3	4.9	4.2	5.2	4.8	4.0	6.6
Nicotinic acid	114	86	144	148	142	27	174	110	171	242	98	134	332	227	126	134	186	138	152
Back "B"																			
Thiamine	6.9	3.9	7.4	7.3	10.6	4.8	7.8	2.8	1.8	8.5	3.3	3.4	6.7	3.5	4.8	9.6	3.9	4.6	5.6
Riboflavin	....	....	....	....	....	....	8.4	7.7	6.5	7.6	7.8	6.9	6.8	3.9	4.5	5.4	2.4	4.3	6.0
Nicotinic acid	146	90	134	152	132	70	207	128	137	183	114	113	276	183	201	198	154	128	153
Back "C"																			
Thiamine	12.8	3.0	5.9	4.9	5.9	4.3	6.2	2.0	1.6	5.4	2.4	3.4	7.0	1.3	3.4	5.4	3.9	3.3	4.5
Riboflavin	....	....	....	....	....	....	9.0	4.9	3.2	8.6	5.4	5.1	4.8	2.8	3.2	4.5	2.4	3.2	4.8
Nicotinic acid	120	94	105	142	158	82	246	150	88	171	116	72	308	151	161	163	154	137	146
Back "D"																			
Thiamine	6.0	3.1	4.0	3.6	6.4	4.2	6.0	2.7	2.6	3.8	2.1	6.1	4.4	2.1	2.4	3.8	3.1	2.7	3.8
Riboflavin	....	....	....	....	....	....	8.0	5.6	4.4	7.7	4.6	6.3	4.1	2.6	2.7	3.6	3.4	2.8	4.6
Nicotinic acid	126	86	136	132	146	94	180	132	82	160	112	90	280	186	126	174	170	120	140
Back "E"																			
Thiamine	5.2	2.8	5.6	5.0	6.2	7.5	8.9	5.8	3.2	4.6	2.6	2.9	6.2	4.2	5.2	6.2	3.8	3.8	5.0
Riboflavin	....	....	....	....	....	....	9.8	6.8	7.2	7.1	7.8	8.2	5.4	3.4	4.2	3.8	3.9	3.5	5.9
Nicotinic acid	84	62	112	84	92	90	189	178	116	162	96	105	222	172	178	138	135	96	128
Back "F"																			
Thiamine	3.8	5.9	5.4	4.1	6.3	5.0	7.4	4.4	3.8	7.4	2.4	3.9	5.4	1.6	4.6	6.6	2.6	2.8	4.6
Riboflavin	....	....	....	....	....	....	9.0	5.4	4.8	8.0	4.3	6.8	5.8	3.6	4.2	3.6	4.4	3.8	5.3
Nicotinic acid	72	72	68	70	93	51	229	103	106	182	114	86	206	128	108	200	127	88	117
Treatment means																			
Thiamine	7.7	3.8	5.3	5.5	7.5	5.1	7.0	3.7	3.0	6.1	2.7	3.8	6.8	2.9	4.1	6.4	3.6	3.5	4.9
Riboflavin	....	....	....	....	....	....	8.8	6.5	5.2	8.1	6.1	7.2	5.7	3.5	3.8	4.3	3.6	3.6	5.5
Nicotinic acid	110	82	116	121	127	69	204	134	117	183	108	100	270	174	150	168	154	118	139
Grouped treatment means																			
Thiamine	.....		5.40		6.30			4.59			4.18			4.61			4.46		.....
Riboflavin	.....		.....		.....			6.84			7.14			4.34			3.82		.....
Nicotinic acid	.....		118.8		98.0			151.5			130.6			198.3			146.6		.....

[d] T., T.C., and C.F. mean thawed, thawed and cooked, and cooked—frozen, respectively.

squares, with their expectations for thiamine, are given in Table 3. The resulting estimates of the components of variance are given in the last column of the table. The percentage of the total variation, which each of these components account for, is given in Table 4, together with those for nicotinic acid and riboflavin.

TABLE 3

Variance component analysis for thiamine content of lamb chops

Source of variation	Degrees of freedom	Mean squares	Expectation of mean square	Components
Total	215	0.04262	$\sigma_e^2+0.995\sigma_t^2+0.949\sigma_l^2+0.949\ \sigma_{tl}^2+0.837\sigma_b^2$	
Backs	5	0.17173	$\sigma_e^2+2\sigma_t^2+36\sigma_b^2$	$\sigma_b^2=0.00417$
Treatments	17	0.28831	$\sigma_e^2+2\sigma_t^2+1.412\sigma_l^2+12\sigma_t^2$	$\sigma_t^2=0.02190$
Backs × treatments	85	0.02746	$\sigma_e^2+2\sigma_t^2+2.118\sigma_{tl}^2$	.....
Locations	17	0.08559	$\sigma_e^2+2\sigma_t^2+12\sigma_l^2+1.412\sigma_{tl}^2$	$\sigma_l^2=0.00275$
Remainder	68	0.01293	$\sigma_e^2+2\sigma_t^2+0.353\sigma_l^2+0.353\sigma_{tl}^2$	$\sigma_{tl}^2=0.00586$
Error	108	0.00990	σ_e^2	$\sigma_e^2=0.00990$

TABLE 4

Percentage of the total variation accounted for by each of the various sources

Source of variation	Percentage of total variance		
	Thiamine	Nicotinic acid	Riboflavin
Backs	9.4	5.8	11.4
Treatments	49.1	61.8	62.5
Locations	6.2	0	0
Interactions	13.1	22.7	19.2
Error	22.2	9.7	6.9

Location differences in nicotinic acid and riboflavin content of lamb chops were essentially zero in these data. Such differences are of only minor importance in the thiamine composition of lamb chops. Little inaccecuracy should, therefore, exist when comparing treatment effects, even though chops from exactly the same location did not receive every treatment.

The mean squares from the completed analysis of variance for each of the three vitamins are given in Table 5. Individual comparisons were made between certain treatment means. Although the comparisons made do not constitute a set of orthogonal comparisons, they are those which were planned from the type of treatments applied.

Differences between backs were highly significant for all three vitamins. The back means, over-all treatments, are shown in the last column of Table 2. The average content of thiamine in the 6 backs varied from 3.8 to 5.9 μg. per gram of tissue; nicotinic acid from 117 to 153; and riboflavin from 4.6 to 6.6. Those backs which were low in one of the vitamins also tended to be low in the other two, and vice versa. Since the 6 ewe lambs in this experiment received the same ration, the differences between backs suggest the possibility of genetic differenees existing in the ability

of sheep to synthesize and store these vitamins in their tissues. However, such differences could also be explained on the basis of environmental effects, which are permanent, but different from one lamb to another.

The differences between treatments were highly significant for each of the three vitamins. In fact, such differences were by far the most important of all sources of variation studied, accounting for from 49 to 62% of the total variation observed. However, the 6 backs did not respond

TABLE 5

Analyses of variances (mean squares only) in thiamine, nicotinic acid, and riboflavin content of lamb chops*

Source of variation	Degrees of freedom	Thiamine	Nicotinic acid	Degrees of freedom	Riboflavin
Total	215	0.04262	0.03129	143	0.02875
Backs	5	0.17173**	0.08763**	5	0.10484**
Treatments	17	0.28831**	0.26344**	11	0.26200**
Fresh					
Raw vs. fried	1	0.45024**	0.08791*	.....	.....
24 Hours vs. 48 hours	1	0.04355	0.15461**	.....	.....
24 Hours					
Raw vs. −15°F.	1	0.08213	0.00304	.....	.....
Raw vs. 0°F.	1	0.07323	0.00795	.....	.....
−15°F. vs. 0°F.	1	0.00026	0.00116	.....	.....
48 Hours					
Raw vs. −15°F.	1	0.00459	0.02534	.....	.....
Raw vs. 0°F.	1	0.10878*	0.32601**	.....	.....
−15°F. vs. 0°F.	1	0.15806*	0.53312**	.....	.....
3 Months vs. 6 months	1	0.01971	0.29094**	1	1.99862**
3 Months					
−15°F. vs. 0°F.	1	0.01911	0.07216*	1	0.01177
6 Months					
−15°F. vs. 0°F.	1	0.00522	0.25767**	1	0.04493*
3 Months and 6 months					
Thawed vs. thawed—cooked	1	2.58248**	0.61948**	1	0.51556**
Thawed vs. cooked—frozen	1	1.78867**	1.32641**	1	0.45708**
Thawed—cooked vs. cooked—frozen	1	0.07268	0.132953**	1	0.00176
Back × treatment	85	0.02746**	0.01723**	55	0.00986**
Error	108	0.00990	0.00321	72	0.00226

*The original data was transformed to logarithms for the statistical analyses.
* Indicates significance at 0.05% level of probability.
** Indicates significance at 0.01% level of probability.

alike on all treatments as shown by the highly significant back × treatment interactions for all three vitamins. There seem to be at least two possible explanations for the back × treatment interactions. One is the possibility that the plus and minus deviations among the errors were correlated for the two determinations on the same sample. A small amount of this may have occurred since the determinations were made by three men and at different times. This would make the error mean square in the analysis of variance too small and the back × treatment interaction mean square too large. The second possibility is that of either genetic or permanent environmental differences between the backs in their ability to retain or synthesize these vitamins under cold and hot treatments.

The pan-fried fresh lamb chops had highly significantly less thiamine (3.8 as compared to 7.7 μg. per gram tissue) than the fresh raw chops. The percentage of decrease in 5 of the backs varied from 43 to 77%. The other back, back "F," showed a slight increase in thiamine content after frying.

There was an average reduction of 25% (110 to 82 μg. per gram of tissue) in the nicotinic acid content of the 6 fresh backs after frying. This difference is statistically signficant at the 0.05% level.

The difference in the thiamine content from 24 to 48 hours of storage was not significant indicating that the difference observed (5.4 at 24 hours as compared to 6.3 at 48 hours of storage) could have occurred by chance. On the other hand, the 20.8 μg. per gram tissue more of nicotinic acid in chops stored for 24 hours than in those stored for 48 hours was highly significant.

No apparent difference occurred in either the thiamine or nicotinic acid content between lamb chops kept at −15°F.(−26°C.) and those kept at 0°F.(−18°C.) for 24 hours of storage. However, differences did exist in the thiamine and nicotinic acid content of chops that were stored at these two temperatures for 48 hours. Chops that were kept at −15°F. for 48 hours had significantly more thiamine and highly significantly more nicotinic acid than did those chops which were kept at 0°F. for the same length of time. The differences between the thiamine content of fresh lamp chops and those frozen and stored for 24 to 48 hours were greater from a percentage standpoint than those between the nicotinic acid content of the same chops. The thiamine content was decreasing while the nicotinic acid content was remaining about the same or increasing slightly.

There was essentially no difference between the thiamine content of lamb chops stored for 3 months and those stored for 6 months. There was also no significant difference between the thiamine content of chops stored at 0°F. and those stored at −15°F. at 3 or 6 months of storage.

The nicotinic acid content of frozen lamb chops increased materially during storage from an average of 110 μg. per gram of tissue for the fresh chops to an average of 270 μg. per gram of tissue for chops stored at −15°F. for 6 months. These differences were all highly significant statistically. It is well known that a breakdown of the tissues continues even during frozen storage. One of the by-products of this breakdown seems to be nicotinic acid. Those chops which were kept at −15°F. contained more nicotinic acid than did those kept at the higher temperature of 0°F. at both 3 and 6 months of storage.

The riboflavin content of lamb chops was highly significantly less when they were frozen and stored for 6 months than if stored for only 3 months. There was no significant difference between the riboflavin content of chops stored at −15°F. and chops stored at 0°F. at these two storage periods.

The thawed, raw lamb chops at 3 and 6 months of storage contained highly significantly larger amounts of each of the three vitamins than the thawed and pan-fried chops or the chops cooked in the frozen state. Thawing and then cooking resulted in retention of more of the nicotinic acid in the chops than when they were cooked in the frozen state. This indicates that during the process of thawing, bacteria and enzymes synthesize nicotinic acid at an accelerated rate.

SUMMARY

Lamb chops from 6 ewe lambs were analyzed for their thiamine, riboflavin, and nicotinic acid content. These chops were subjected to 18 combinations of freezing, cooking, and storage treatments.

Differences between backs were highly significant in their content of all three vitamins. The greatest differences occurred in the riboflavin content of the six backs.

The total differences between the 18 treatments were highly significant for each of the three vitamins. Different backs did not respond alike to all treatments for either of the three vitamins.

The decrease in thiamine and nicotinic acid content from cooking fresh lamb chops was approximately 50 and 25%, respectively.

The thiamine content was decreasing up to 48 hours of frozen storage, while the nicotinic acid content was remaining about the same or increasing slightly.

There was no significant difference between the thiamine content of chops stored for 3 or 6 months. However, a decrease of 1.2 μg. per gram of tissue occurred up to 3 months of storage. The nicotinic acid content, on the other hand, increased 145 and 53% during the 6 months of freezer storage at −15°F. and at 0°F., respectively.

Lamb chops lost more of their riboflavin content when they were frozen and stored for 6 months than when stored for only 3 months. The amount of riboflavin lost was about the same in chops stored at −15°F.(−26°C.) as in chops stored at °F.(−18°C.).

Acknowledgment

We wish to acknowledge the assistance of Mrs. June Anderson, Mrs. Sybil Brislain, and Mr. W. V. Hartwell with the vitamin analyses.

LITERATURE CITED

1. Campbell, Rita, Hiltz, Mary C., and Robinson, Allen D. The thiamin content of meat. *Can. J. Research*, **F**, **24**, 140 (1946).
2. Darby, W. J., and Day, P. L. Riboflavin content of meats. *J. Nutrition*, **16**, 209 (1938).
3. Kellogg, M. Vitamin B in lamb tissues and organs. S. Dakota Agr. Expt. Sta., *Bull.*, **306** (1936).
4. *Meat Manual*, 1945. The National Livestock and Meat Board, Chicago, Ill.
5. Michelsen, O., Condiff, H., and Keyes, A. The determination of thiamin in urine by means of the thiochrome technique. J. Biol. Chem. **160**, 361 (1945).
6. Snell, E. E., and Strong, F. M. A microbiological assay for riboflavin. *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939).
7. Snell, E. E., and Wright, L. D. A microbiological method for the determination of nicotinic acid. *J. Biol. Chem.*, **139**, 675 (1941).
8. Waisman, H. A., and Elvehjem, C. A. *The Vitamin Content of Meat*, 1941, Burgess Publishing Co., Minneapolis, Minn.

ASCORBIC ACID CONTENT OF SOME MISCELLANEOUS TROPICAL AND SUBTROPICAL PLANTS AND PLANT PRODUCTS[a]

MARGARET J. MUSTARD
Tropical Food Research Laboratories, University of Miami, Coral Gables, Florida

(Manuscript received April 21, 1951)

A large number of tropical and subtropical plants are grown in southern Florida where some have attained commercial importance and others are used chiefly as dooryard plantings.

Numerous investigations have been conducted in this country and elsewhere that show the dietary value of these plants and their products. Many of these tropical and subtropical plants have been found to contain large amounts of ascorbic acid. The guava, mango, papaya, and Barbados cherry are but a few of the tropical fruits which are now recognized as excellent sources of this vitamin.

The purpose of the present paper is to present additional information pertaining to the ascorbic acid content of some miscellaneous tropical and subtropical plants and plant products of southern Florida. Since many of these are not widely known in this country, a brief description of the plants and their uses is included.

DESCRIPTION OF PLANTS AND THEIR USES

The Barbados cherry, *Malpighia punicifolia*, Linn., is a spreading shrub bearing small red fruit resembling the surinam-cherry. The flavor of the fruit varies from sour to almost sweet. They can be used in numerous ways including frozen or heat processed punch bases, in ice-cream and ice-cream toppings, in fruit purées, and in jellies.

The breadnut, *Brosimum alicastrum*, Swartz, is also known as the Ramon tree of Yucatan. The leaves and seed pods of this plant are used as fodder *(3)*.

The calamondin, *Citrus mitis*, Blanco, is an acid species of citrus which makes it well adapted for use in beverages *(3)*.

The carambola *Averrhoa carambola*, Linn., bears fruit which are golden yellow in color, longitudinally ribbed, and approximately three to five inches in length. They can be eaten fresh or used in the preparation of jellies, preserves, or beverages *(15)*.

The pummelo, *Citrus grandis*, Osbeck, is a large fruit resembling the grapefruit in appearance. The pulp of this fruit is more solid than the grapefruit and the juice vesicles can be readily separated from one another *(19)*. The juice vesicles of this fruit are used in salads.

The canistel or eggfruit, *Lucuma nervosa*, A. DC., bears fruit the flesh of which resembles in appearance the yolk of hard boiled eggs, is very sweet and somewhat musky *(17)*. The canistel can be eaten out of hand, served with butter and lime juice, with cream and sugar, or used in pies and puddings *(14)*.

Gold Coast jasmine, *Jasminum dichotomum*, Vahl, is a climbing shrub bearing white fragrant flowers and black juicy fruit *(8)*.

The jaboticaba, *Myrciaria cauliflora*, Berg., bears dark purple fruit which are produced on the trunk of the tree. The fruit are similar to the muscadine grape and like the grape can be eaten out of hand or used in the preparation of jellies, juices, and wines *(15)*.

---

[a] The work here reported was carried out while the author was a member of the Botany Department.

The jelly palm, *Butia capitata*, Becc. var. *Nehrlingiana*, bears small, yellow, egg-shaped fruit which are used in jelly making *(10)*.

The Key, Mexican, or West Indian lime, *Citrus aurantifolia*, Swingle, is the small commercial lime cultivated in most of the citrus sections throughout the world *(19)*.

Khat, *Catha edulis*, Forsk., is a plant of commercial importance in Arabia where the young twigs are chewed and the leaves used in the preparation of a beverage by the Arabs. The use of khat is an ancient custom in that country where the inhabitants claim it has stimulative and recuperative properties. The plant has been successfully introduced into southern Florida but no stimulating effects have been noted from its use in this country *(4, 6, 7)*.

Kumquats, *Fortunella margarita*, Swingle, are small, oblong orange colored citrus fruit about one and one-half inches long *(3)*. They can be eaten fresh, candied, or made into jams and marmalades.

The lingaro, *Elaegnus philippensis*, Perr., is a climbing or scandent evergreen shrub grown in southern Florida which unsupported attains a height of ten feet. The fruits are about the shape of a very small olive and pink or pale red in color. Lingaros may be eaten out of hand or used in jelly making *(15)*.

The myrobolan, *Phyllanthus emblica*, Linn., is a shrub or small tree grown in the warmer sections of Florida. The fruit is similar in appearance to the otaheite-gooseberry, another member of the same genus. It is utilized either raw or preserved *(4, 15)*.

Pineapples, *Ananas comosus*, Merr., have been grown commercially in Florida for many years. The Abachi and Smooth Cayenne are two of the commercial varieties.

The rose-apple, *Eugenia jambos*, Linn., is a large spreading ever-green tree. The yellow to pinkish white fruit may be eaten fresh but are recommended for use as a candied or preserved product or for jellies *(15)*.

The roselle, *Hibiscus sabdariffa*, Linn., is grown as a dooryard plant in southern Florida. The deep red calyces of this plant are used in the preparation of sauces, jellies, jams, and wine *(14)*.

Sapodillas, *Achras zapota*, Linn., are considered by Popenoe *(17)* as one of the best fruits of tropical America. The fruit may be round, oval, or conical in shape and average two to three and one-half inches in diameter. The thin, rusty brown, somewhat scurfy skin resembles that of the Irish potato. The yellow-brown flesh of the ripe fruit is sweet and rich in flavor. Sapodillas are used chiefly as a dessert fruit.

The surinam-cherry or pitanga, *Eugenia uniflora*, Linn., is grown in southern Florida as a hedge plant or ornamental shrub. The flesh of the fruit is sub-acid in flavor, soft, and juicy. The fruits can be eaten fresh or used in the preparation of jellies and beverages *(17)*.

The wampee, *Clausena lansium*, Skeels, is a "vase shaped" tree branching about a foot above the ground. Dr. Fairchild has described the fruit as having a "sweet oriental flavor" and lacking the "pucker" of Concord grapes. This tree seems well adapted to cultivation in Florida *(9)*.

White sapotes, *Casimiroa edulis*, Llav. and Lex., can be grown in south Florida. The flesh of the fruit is yellowish to white in color and varies in flavor from sweet to slightly bitter. They can be eaten fresh or preserved *(17)*. The fruits analyzed were taken from a seedling of the Sarah Jones. They were small but had no bitter flavor.

Gnetum gnemon, Linn., is a native of Malay. The seeds of this plant are eaten in Amboina fried, boiled, or roasted and the leaves are cooked and eaten as spinach *(18)*.

Materials and Methods

The plant materials used in this investigation were grown under diversified cultural conditions as they were obtained from local dooryard plantings, nurseries, and botanic gardens.

All of the determinations, with the exception of those of the calamondin and kumquat were made by the method of Loeffler and Ponting *(12)*, modified to use a calibration curve instead of the formula recommended by these workers for converting the colorimeter readings into mg. of ascorbic acid per 100 g. of material. The kumquats and calamondins were analyzed by essentially the same method as that described by Heinze *(11)*. Due to the wide variation in ascorbic acid content, it was necessary to use various sized samples ranging from 5 to 25 g. A description of the portion of each

TABLE 1

Ascorbic acid content of some miscellaneous tropical and subtropical plants and plant products

Common name	Scientific name	Description of sample	No. of analyses	Ascorbic acid content		
				Average	Range	
					Minimum	Maximum
				mg./100 g.	*mg./100 g.*	*mg./100 g.*
Barbados cherry	*Malpighia punicifolia*, Linn.	Sweet, ripe fruit, peel, and pulp	6	959.0	860.0	1,135.0
		Jelly stored six months	15	335.2	271.0	450.2
Breadnut	*Brosimum alicastrum*, Swartz	Leaves	2	70.8	56.6	85.0
Calamondin	*Citrus mitis*, Blanco	Juice	4	30.8	30.0	31.5
		Whole, ripe fruit	5	101.0	88.4	111.3
		Skin	3	159.1	130.0	173.9
Carambola	*Averrhoa carambola*, Linn.	Whole, ripe fruit	4	86.4	77.1	95.5
Egg fruit	*Lucuma nervosa*, A. DC.	Ripe fruit, peel, and pulp	2	49.2	37.2	61.2
		Ripe fruit, peel, and pulp	3	104.0	94.0	110.0
Gold Coast jasmine	*Jasminum dichotomum*, Vahl	Unripe, whole fruit	3	8.8	8.2	9.8
Jaboticaba	*Myrciaria cauliflora*, Berg.	Whole fruit	2	29.0	27.3	30.7
Jelly palm	*Butia capitata*, Becc. var. *Nehrlingiana*	Peel and pulp	4	83.3	74.1	90.6
		Jelly	2	14.0	12.4	15.6
Key lime jelly	*Citrus aurantifolia*, Swingle	Freshly prepared jelly	15	3.3	2.0	4.9
Khat	*Catha edulis*, Forsk.	Leaves and branch tip	5	135.7	127.5	152.5
		Leaves	1	324.6	.....	.....
Kumquat	*Fortunella margarita*, Swingle	Whole, ripe fruit	4	52.7	52.0	53.5
		Skin	4	102.8	94.5	105.8
Lingaro	*Elacagnus philippensis*, Perr.	Whole, ripe fruit	3	6.8	6.5	7.4
Myrobolan	*Phyllanthus emblica*, Linn.	Whole fruit	7	1,561.1	1,220.0	1,814.0
Pineapple	*Ananas comosus*, Merr.					
Abachi		Pulp, ripe fruit	5	96.3	91.4	99.0
Smooth Cayenne		Pulp, ripe fruit	5	24.4	22.3	26.0
Pummelo	*Citrus grandis*, Osbeck	Skin	2	98.5	92.0	105.0
		Flesh	2	40.2	40.0	40.3
Rose-apple	*Eugenia jambos*, Linn.	Pulp and peel, ripe fruit	3	28.3	27.1	29.8
Roselle	*Hibiscus sabdariffa*, Linn.	Calyces	2	12.9	12.0	13.8
Sapodilla	*Achras zapota*, Linn.	Pulp, ripe fruit	4	14.2	11.0	15.8
Surinam-cherry	*Eugenia uniflora*, Linn.	Red, ripe fruit, peel and pulp	4	38.5	33.9	43.9
		Red, unripe fruit, peel and pulp	1	39.4	.....	.....
		Black, ripe fruit, peel and pulp	2	25.3	25.3	25.3
		Jelly, freshly prepared from red fruit	15	14.2	12.3	18.0
Wampee	*Clausena lansium*, Skeels	Pulp, ripe fruit	2	28.8	28.3	29.2
White sapote	*Casimiroa edulis*, Llav. & Lex.	Pulp, ripe fruit	4	40.7	32.5	44.0
	Gnetum gnemon, Linn.	Leaves	1	324.6	.....	.....

plant analyzed is given in Table 1. A longitudinal section was removed from each of the larger fruit for analysis; whereas it was necessary to use a composite sample consisting of the edible portions of several fruit for the analysis on the smaller ones. Except for a few fruit which were allowed to ripen at room temperature, all of the samples were stored under refrigeration for a period of from one to two days until analyzed.

RESULTS AND DISCUSSION

It is apparent from the results in the accompanying table that several of these tropical and subtropical plants provide excellent supplementary sources of ascorbic acid.

Since the two varieties of pineapple described in Table 1 were obtained from the same plantation where the plants received similar cultural treatments, it seems reasonable to assume the difference in ascorbic acid content can be considered a varietal and not an environmental difference.

The results in the accompanying table show that the carambola is a good source of ascorbic acid.

The ascorbic acid content of jelly prepared from the jelly palm was found to compare favorably with that of orange marmalade *(5)*.

Depending of course upon the quantity consumed, the ancient custom of khat chewing in Arabia may be inadvertently supplying the people of that country with some of their daily requirement of ascorbic acid.

Apparently there is a considerable variation in the ascorbic acid content of egg fruit since fruit picked from one tree were found to average 49.2 mg. per 100 g. of fruit and those of another tree were found to average 104.0 mg.

Previous determinations have shown that the sour, ripe Barbados cherry grown in Florida contains from 1,579 to 2,712 mg. of ascorbic acid per 100 g. of fruit *(16)*; the ripe "agreeably acid" fruit of Puerto Rico contains from 1,030 to 2,700 mg. *(1)*; and the juice of the ripe fruit of Puerto Rico contains as high as 1,522 mg. per 100 ml. *(2)*. Although the sweet type does not have as high an ascorbic acid content as the more sour type, it is nevertheless an excellent source of this vitamin. Barbados cherry jelly was found to decrease in ascorbic acid content during storage. After six months of storage at room temperature, this jelly was found to contain 335 mg. as compared to the original value of 599 mg. for freshly prepared jelly *(16)*. The ascorbic acid content of Barbados cherry jelly is outstanding compared to that of other jellies and marmalades. Orange jelly contains less than 1 mg. of ascorbic acid *(5)*; orange marmalade from less than 1 to 14 mg. *(5)*; and guava jelly 53 mg. per 100 g. of product *(13)*.

Florida grown myrobolans were found to be an exceptionally rich source of ascorbic acid. The value shown in Table 1 is considerably higher than that previously found by Giri and Doctor of India referred to in a recent article by Asenjo and Freire De Guzman *(1)*.

Other fruits containing relatively high amounts of ascorbic acid include the white sapote, pummelo, calamondin, surinam-cherry, kumquat, jaboticaba, rose-apple, and wampee.

Although many of these tropical and subtropical plants are not widely cultivated in Florida at the present time, some of those having exceptionally high ascorbic acid contents seem to merit further investigation pertaining to their cultivation and utilization.

SUMMARY

The ascorbic acid content of a number of miscellaneous tropical and subtropical plants and plant products is presented. The myrobolan, Barbados cherry, Abachi pineapple, carambola, and khat were found to contain exceptionally large amounts of ascorbic acid.

Acknowledgment

The author gratefully acknowledges her indebtedness to the U. S. Plant Introduction Garden, Coconut Grove, Florida; Fairchild Tropical Garden, Coconut Grove, Florida; Dr. David Fairchild, Coconut Grove, Florida; and others who contributed the materials used in this study.

LITERATURE CITED

1. Asenjo, Conrado F., and Freire De Guzman, Ana Rosa. The high ascorbic acid content of the West Indian cherry. *Science*, **103**, 219 (1946).
2. Asenjo, Conrado F., and Moscoso, G. Carlos. Ascorbic acid content and other characteristics of the West Indian cherry. *Food Research* **15**, 103 (1950).
3. Bailey, L. H., and Bailey, Ethel Zoe. *Hortus Second*. 1941, The Macmillan Co., New York, N. Y.
4. Bailey, L. H., and Bailey, Ethel Zoe. *The Standard Cyclopedia of Horticulture*. 1947, The Macmillan Co., New York, N. Y.
5. Booher, Lela E., Hartzler, Eva R., and Hewston, Elizabeth M., 1942. A compilation of the vitamin values of foods in relation to processing and other variants. U.S.D.A. *Cir.* 638.
6. Fairchild, David. *Exploring for Plants*. 1930, The Macmillan Co., New York, N. Y.
7. Fairchild, David. *The World Was My Garden*. 1943, Charles Scribner's Sons, New York, N. Y.
8. Fairchild, David. *The World Grows Round My Door*. 1947, Charles Scribner's Sons, New York, N. Y.
9. Fairchild, David. The Wampee, a Fruit Tree of the Far East. Fla. Plant Immigrants, Fairchild Tropical Garden. *Occasional Paper* 19 (1950).
10. Hawkes, Alex. D. The major kinds of palms. *Botanical Papers Fairchild Tropical Garden*, No. II (1950).
11. Heinze, P. H., Kanapaux, Margaret S., Wade, B. L., Grimball, P. C., and Foster, Ruth L. Ascorbic acid content of 39 varieties of snap beans. *Food Research*, **9**, 19 (1944).
12. Loeffler, H. J., and Ponting, J. D. Ascorbic acid—rapid determination in fresh, frozen, or dehydrated fruits and vegetables. *Ind. Eng. Chem., Anal. Ed.*, **14**, 846 (1942).
13. Miller, Carey D., Louis, Lucille, and Yanazawa, Kisako. Vitamin values of Hawaiian grown fruits and vegetables. Hawaii Agr. Expt. Sta., *Progress Notes*, **36** (1944).
14. Morton, Kendall P., and Morton, Julia. *Fifty Tropical Fruits of Nassau*. 1946, Text House, Coral Gables, Florida.
15. Mowry, Harold, Toy, L. R., and Wolfe, H. S. Miscellaneous tropical and subtropical Florida fruits. Univ. Florida Agr. Expt. Sta., *Bull.*, **109** (1941).
16. Mustard, Margaret J. The ascorbic acid content of some malpighia fruits and jellies. *Science*, **104**, 230 (1946).
17. Popenoe, Wilson. *Manual of Tropical and Subtropical Fruits*. 1920, The Macmillan Co., New York, N. Y.
18. Sturtevant, E. Lewis. *Sturtevant's Notes on Edible Plants*. 1919, Edited by U. P. Hedrick, J. B. Lyon Co., Albany, N. Y.
19. Webber, Herbert John, and Batchelor, Leon Dexter. *The Citrus Industry, I*. 1946, University of California Press, Berkeley and Los Angeles, California.

ROLE OF PECTIN AS RELATED TO DEHYDRATION AND REHYDRATION OF SIMULATED FRUIT PREPARATIONS[a,b,c]

GEORGE L. BAKER, JOHN F. KULP, AND RALPH A. MILLER
Department of Chemistry, University of Delaware Agricultural Experiment Station, Newark, Delaware

(Manuscript received June 7, 1951)

During war periods there is an exceptionally heavy demand for dehydrated fruit and vegetable products. This is due to the economies of transportation and storage of dried foods accounting for only a fraction of the volume and weight of fresh or canned products. However, improvements of texture, appearance, and palatability of rehydrated food products are needed. Such improvements would aid in the more ready acceptance of dehydrated fruits and vegetables by military personnel.

A preliminary study *(1)* indicated a correlation between pectic substances naturally present in the vegetative tissues of dehydrated fruits and vegetables and the quality of these products. These findings suggested that fundamental studies upon simulated dehydrated products made up of cellulose, sugars, and some of the various components naturally found in fruits and vegetables might aid in understanding the effect of each component during dehydration and rehydration. Initial work of this nature showed that pectins are probably the most important class of substances affecting rehydration and that their action varied with the degree of methylation. In view of these results, more extensive studies were undertaken to determine the role of pectins as related to dehydration and rehydration of "synthetic" fruit preparations. Since it has been indicated that the degree of methylation of the pectins greatly affected rehydration characteristics, pectins of varying degrees of methylation were produced in the simulated fruit products. This was accomplished by the use of the enzyme pectin-methylesterase in situ previous to dehydration. In addition, applications of the findings were considered in a practical manner upon apricots. A brief review of the work is presented at this time as a possible stimulus to further search for experimental methods for use toward the overall objective, namely, the improvement of the rehydration properties of dehydrated foods for enhancement of texture, flavor, appearance, and palatability.

PROCEDURE

Preparation of experimental samples. The simulated fruit products were made up with alpha-cellulose fibers serving as the basic ingredient and active swelling agent for

[a] The subject matter of this paper has been undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and had been assigned No. 310. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsements of the Department of the Army.

[b] Published as Miscellaneous Paper No. 119 with the approval of the Director of the Delaware Agricultural Experiment Station.

[c] Presented at the Boston meeting of the A.C.S., April, 1951.

all samples. The cellulose fibers were prepared by grinding a high grade filter paper in a hammer mill to pass a 1.27-mm. slit screen. Johns Manville "Hyflo Super-Cel" filter aid was used as the chemically inactive diluent which would allow controlled variation of the percentages of the various ingredients. Four grams of the cellulose fibers were used in each of the experimental samples; that is, each dehydrated product contained 20% cellulose. The simplest product was the one consisting of 20% cellulose and 80% filter aid.

Other ingredients used in the experimental dehydrated products were citrus pectin (188 grade, 11.6% CH_2O), sucrose, dextrose, sodium di-hydrogen phosphate, disodium hydrogen phosphate, citric acid, and calcium chloride. These were used in varying amounts which necessitated the amount of filter aid being adjusted so that the total dry weight of each sample always amounted to 20 g.

In preparing the samples all soluble ingredients were mixed and added to 30 ml. of water. The pectin was wet with 95% ethyl alcohol (2 ml. per g.) and then dispersed in 50 ml. of water. The 30 ml. of solution containing the soluble ingredients was then added to the 50 ml. of dispersed pectin, mixed and heated to 100°C. Finally, the cellulose with which the necessary Super-Cel had previously been mixed was added and the total weight adjusted to 100 g. The product was spread on an 8-inch watch glass and dried at 60°C. to constant weight. This was usually attained in 24 hours. The dried preparation was run through a hammer mill (Mikro-Samplmill) using a round ⅛-inch pore size screen and then redried at 60°C. to constant weight before placing in an air-tight storage container.

Measurement of rehydration swelling. In rehydration swelling as measured in these experiments, it was assumed that the soluble ingredients should activate the swelling of the insoluble cellulose fibers in a manner somewhat similar to that occurring in the rehydration of natural dried products.

Swelling was measured in graduated hematocrit tubes (6 ml.) as follows: A sample (usually 0.5 g.) of the dehydrated product was placed in a tube held in a vertical position on a counter balance. It was then packed uniformly by tamping with a glass rod against a counterpoised weight of 1500 g. The initial volume occupied by the sample was recorded. Then 6 ml. of water was added for rehydration, the sample thoroughly mixed with this water, allowed to stand for 5 minutes, and then the tube and contents were centrifuged for 2 minutes at a speed of 1600 r.p.m. The difference in volume occupied by the cellulosic fraction was recorded as the effect on swelling due to 5 minutes of rehydration. After thorough stirring, the centrifuging procedure, etc., was repeated at intervals of 15, 30, and 60 minutes. Following the last reading, the solution was poured off and the tubes and contents were weighed to determine the weight of solution absorbed by the cellulose. Swelling was calculated in ml. per g. of cellulose and retention or absorption of solution as g. per g. cellulose. Swelling was measured at 26°C. and 90°C. Determinations were made in triplicate.

RESULTS AND DISCUSSION

Effect of various ingredients upon rehydration swelling of cellulose. In order to determine the effect of each of the various ingredients in the simulated fruit products, each separate ingredient was added, in turn, to the basic quantity of cellulose (4 g.) and sufficient Super-Cel added to adjust total solids to 20 g. Rehydration swelling of the basic mixture composed of 20% cellulose and 80% Super-Cel was 11.7 ml. per g. cellulose at 26°C. and 10.9 ml. per g. at 90°C.

Citric acid, after a slight initial increase, reduced rehydration swelling of the cellulose as shown in Figure 1.

Sodium citrate, used as one of the buffer salts in the final synthetic fruit product, reduced rehydration swelling of the cellulose more than did the citric acid at 26°C. (Figure 2) but showed little effect at 90°C. Sodium dihydrogen phosphate, even when only 2.5% was added to the mixture, had a more drastic effect, reducing the rehydration swelling to 9.3 ml. per

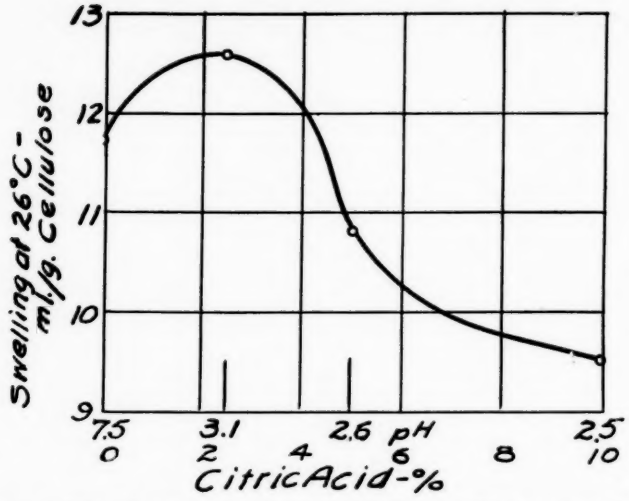


Figure 1. Effect of citric acid on rehydration swelling at 26°C. of dehydrated cellulose, citric acid, and filter aid mixtures.

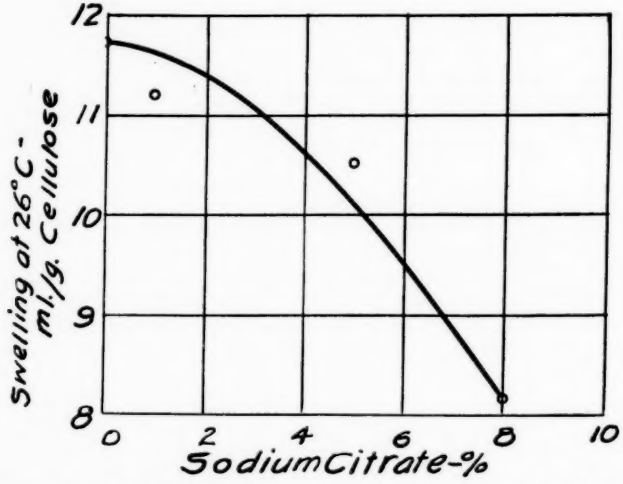


Figure 2. Effect of sodium citrate on rehydration swelling at 26°C. of dehydrated cellulose, sodium citrate, and filter aid mixtures.

g. cellulose at 26°C. and 7.3 ml. per g. at 90°C., pH 5.9. Disodium hydrogen phosphate reduced rehydration swelling at 26°C. to about the same extent as the dihydrogen salt but had little effect at 90°C. The effect of buffers upon rehydration swelling of the cellulose in a pH range of 3 to 7 varied from negligible to a reduction of 80% of swelling capacity as compared with that of the control sample.

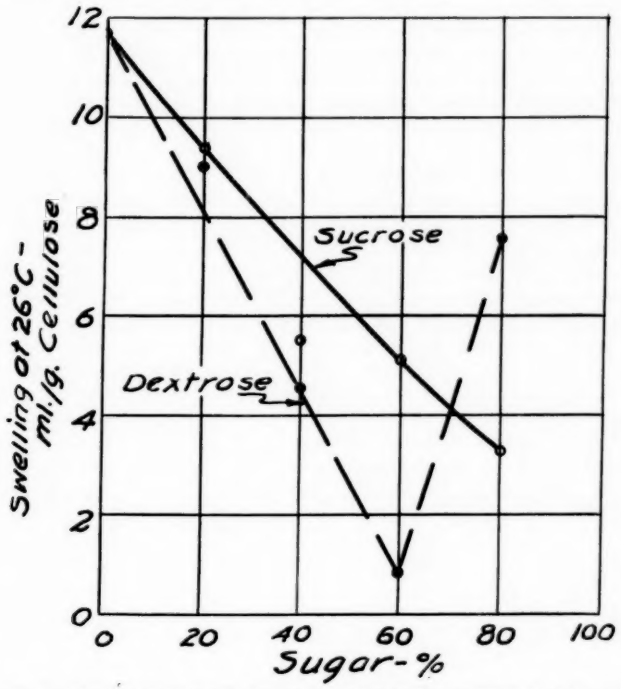


Figure 3. Effect of sucrose and dextrose on rehydration swelling at 26°C. of dehydrated cellulose, sugar, and filter aid mixtures.

Dextrose and sucrose, which normally make up about 50% of a dehydrated product, were found to have a strong depressing effect upon rehydration swelling when they made up less than 60% of the mixture. A larger quantity of dextrose reversed this tendency, however. The effect of sugars at pH 6-7 is shown in Figure 3. If dextrose and sucrose were mixed and added to the cellulose, then rehydration swelling tended to follow the trend established for dextrose alone. If in addition to the sugars, the buffer salts were added in small amounts, as 1 to 2%, the trend was more uniform but the depressing effect upon rehydration swelling was still the same. This held whether working near neutrality (pH 6-7), or with acid present, at pH 2.6-3.3.

When high-methoxyl citrus pectin (188 grade, 11.6% CH_3O) was added to the cellulosic mixture (always containing 20% cellulose) and the Super-Cel accordingly reduced, the dehydrated product became more bulky. Due to this bulkiness at the 10% pectin level, only one-half as much of the dehydrated product (0.25 g.) could be used in the tests for rehydration swelling. Rehydration swelling of the cellulose was reduced at 26°C. when cellulose, pectin, and Super-Cel only were present in the mixture (Figure 4).

Synthetic dehydrated fruit product. Following the study of the specific effects of various individual ingredients on the rehydration swelling of the cellulose, several ingredients were added to the cellulose at the same time in order to build up a simulated fruit product by degrees and obtain group effects. This produced a large amount of data which can be merely summarized at this time.

When all of the buffer salts were combined and added to the cellulose in approximately the same quantities in which they are found in nature in an average fruit product, the net result was more of a decline in swelling than when each was added separately. This decline was accentuated slightly by the presence of acid, but was greatly increased by the presence of sucrose or dextrose, either separately or combined in varying ratios. If acid was added to the mixture of salts and sugars with the cellulose in a proportion similar to that found in fruits, then the rehydration swelling of the cellulose was practically nil. At certain acidities, for instance when enough acid was used to give a mixture at pH 3.3, rehydration swelling was even negative at 26°C. Calcium salts, as for example calcium chloride at 0.25%, made the swelling values even more negative at 26°C. and for the first time caused the cellulose to display negative swelling at 90°C. However, all of these mixed products showed a certain amount of retention of solution by the cellulose although this retention was reduced by about 75% below that of the simplest cellulose-filter-aid mixture. About 5 g. per g. cellulose retention of solution was found in the complex mix against about 22 g. per g. cellulose for that of the simplest mixture at both 26° and 90°C.

When high-methoxyl pectin was introduced into the "synthetic" fruit mixture in combination with the salts, acids, and sugars, the bulkiness of the dehydrated product, previously noted when pectin and basic mix only were used, was no longer accentuated. The most noteworthy effect of introducing other ingredients along with the pectin was the addition of the sugars in proportions of 20 to 60% of the dehydrated product. All concentrations of either dextrose or sucrose, with or without citric acid, reduced rehydration swelling of the cellulose below that of the simplest mixture studied which consisted of 20% cellulose and 80% filter aid.

DEMETHYLATION OF PECTIN IN SYNTHETIC PRODUCTS WITH PECTIN-METHYLESTERASE IN SITU AND ITS EFFECT UPON REHYDRATION SWELLING

Due to the complexity of the data it was difficult to choose any one complete mixture of cellulose, sugars, salts, acid, and pectin that had special properties during dehydration or rehydration which would recommend it for study of the effects of the degree of methylation of the pectin.

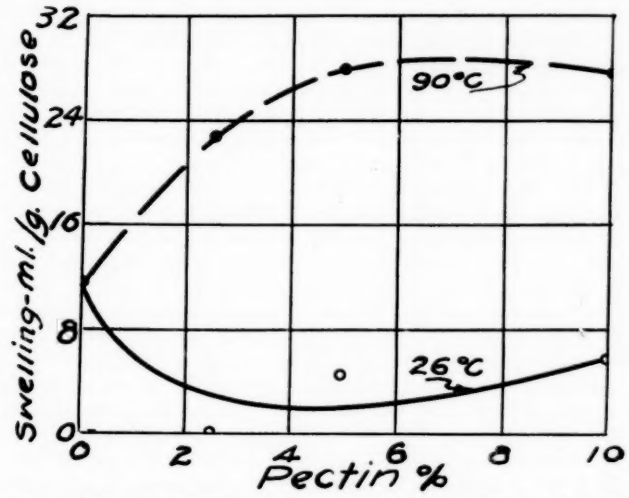


Figure 4. Effect of high-ester pectin on rehydration swelling at 26°C. and 90°C. of dehydrated cellulose, pectin, and filter aid mixtures.

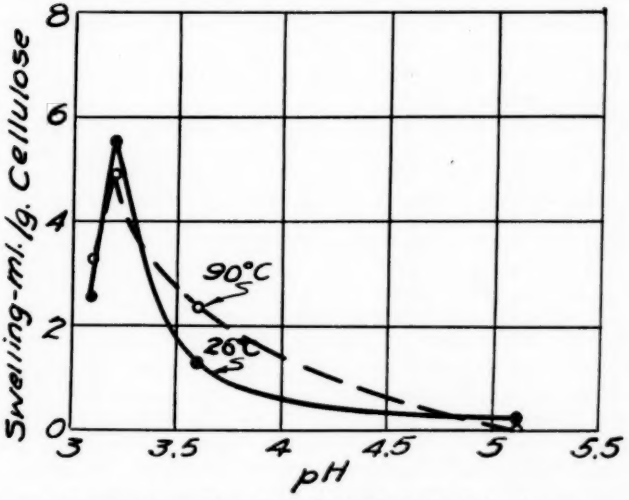


Figure 5. Rehydration swelling of simulated dehydrated fruit mixtures at various acidities at 26°C. and 90°C.

It was decided, therefore, to take an average analysis of miscellaneous fruits *(4)* considering only the ingredients whose effect on the swelling of the cellulose had been investigated. The chosen mixture (dry basis) contained separate ingredients in fixed proportions, except for citric acid, in accord with the following: 20% cellulose, 20% sucrose, 40% dextrose, 1% sodium citrate, 0.5% sodium dihydrogen phosphate, 0.5% disodium hydrogen phosphate, 10% citrus pectin (188 grade, 11.6% CH_3O), and Super-Cel to 100%. This chosen mixture was investigated at various pH values, namely, 5.1, 3.6, 3.2, and 3.1, and the results shown in Figure 5 were obtained. At pH 5.1, almost no swelling of the cellulose was found. It was obvious from past studies *(5)* that the sugar-pectin relationships affected the dispersibility of the high-methoxyl pectin and hence also the rehydration swelling of the cellulose. This nearly negligible rehydration swelling might be considered beneficial in that it would serve as a good reference point to study the effect of demethylation of the pectins.

A simulated fruit product containing the ingredients listed in the chosen mixture given above was used at pH 3.6 in order to study the effects of varied methoxyl content of the pectin upon rehydration swelling. Previous to dehydration the liquid mixture would, of course, contain 80% water and the percentages of ingredients as listed would be one-fifth of that shown. The liquid mixture produced the physical appearance of a puréed fruit product. In addition to listed ingredients, 0.4% of a commercial pectin-methylesterase (obtained through the courtesy of the Rohm and Haas Company of Philadelphia, Pa.) was added to the simulated puréed fruit mixtures and demethylation was allowed to proceed for 6-, 12-, 18-, and 24-hour periods on separate mixtures. At the end of the demethylation period, the enzyme was inactivated by heating the mixture to 80°C. and then the product was dried at 60°C. The dried product was run through the hammer mill, redried to constant weight, and rehydration swelling was measured as before.

Under the conditions of the experiment, rehydration swelling reached a maximum when the pectin present had 4.7% CH_3O (based on a pectin of 100% calcium pectate content). The methoxyl content was determined by the saponification method of von Fellenberg *(3)* and the calcium pectate was based on the method of Emmett and Carré *(2)*, using samples of pectin extracted with boiling water from the dehydrated products and precipitated with isopropyl alcohol. The pH of the treated products after rehydration was 3.6. The viscosity of the isolated liquid portions at pH 3.6 decreased with demethylation, indicating that depolymerization had also taken place. The results obtained are shown in Table 1 and Figure 6.

Enzymic demethylation was retarded by increasing the acidity from pH 3.6 to pH 2.9. As shown in Figure 6, this change in pH caused a slightly different pattern in rehydration swelling. This was possibly due to well-known acid precipitation tendencies of the more demethylated pectins which is illustrated by the increase in viscosity of the isolated liquid portions at pH 2.9 shown in Table 1. Small amounts of calcium, added as calcium chloride, aided rehydration swelling but only slightly. Under conditions of the experiment, wetting agents proved of no value.

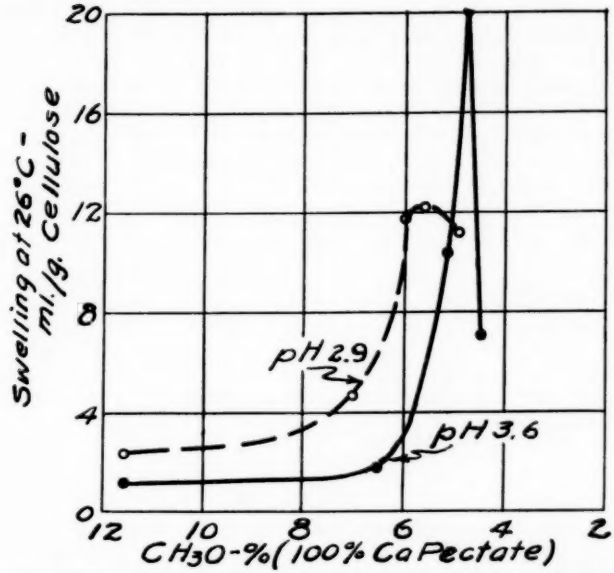


Figure 6. Effect of altering the methoxyl content of the pectins in simulated dehydrated fruit mixtures upon rehydration swelling at 26° C., pH 3.6 and 2.9.

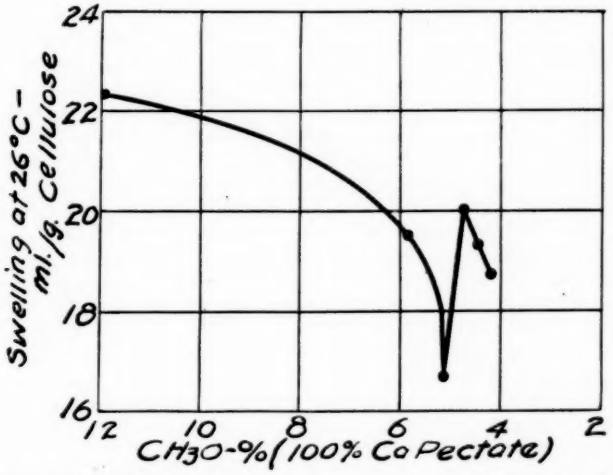


Figure 7. Effect of the methoxyl content of the pectins in dehydrated apricots upon the rehydration swelling at 26°C.

These studies upon the effect of the degree of methylation of the pectins implied that methylation plays a major role in the reconstitution of dehydrated fruits and vegetables and that possibly a product could be developed which would rehydrate easily with relatively cool water. The findings suggest that greater rehydration capacity might be obtained through regulation of the methoxyl content of the pectins in a fruit product, thus a study of the effect of partial demethylation of the pectins in fresh apricots was undertaken.

TABLE 1

Effect of methoxyl content of pectins in a "synthetic" fruit mixture upon rehydration swelling

Length of enzyme treatment	Swelling of cellulose at 26°C.		Pectin, average CH_3O in pectin		Relative viscosity (Ostwald) of drained liquid	
	pH 3.6	pH 2.9	pH 3.6	pH 2.9	pH 3.6	pH 2.9
hours	*ml. per g.*	*ml. per g.*	*%*	*%*		
0	0.2	2.3	11.6	11.6	9.3	5.1
6	1.9	4.7	6.5	7.0	10.3	13.4
12	10.4	11.8	5.1	6.0	5.6	18.4
18	20.1	12.2	4.7	5.6	4.1	27.7
24	7.1	11.2	4.4	4.9	2.4	36.4

Demethylation of pectins in fresh apricots. Fresh apricots were sliced and then rapidly heated to 80°C. with stirring in order to inactivate enzymes which might either depolymerize or deesterify the natural pectins. They were then made into a puréed mass, using a Waring Blendor. Six 100-g. samples of puréed apricot were prepared. One sample was used as a control. To each of the other 5 samples 0.4 g. of pectin-methylesterase was added, and the enzyme allowed to act at 40°C. for 3, 6, 12, 18, and 24 hours, respectively. At the end of the reaction period the puréed sample was heated to 100°C. to inactivate the enzyme. It was then dehydrated at 60°C., milled, and the rehydration swelling evaluated in the manner used previously in the study of model mixtures.

Rehydration swelling at 26°C. of these samples of dehydrated fresh apricot tissues decreased from an initial high for the control of 22.3 ml. per g. cellulose to 16.7 ml. per g. cellulose for 6 hours of enzyme treatment and then increased. Samples were tested in triplicate and the average value taken. Apparently too much enzyme was used under the condition of the experiment because the methoxyl contents (based on 100% calcium pectate) decreased from 11.85% for the control to 5.80% for the 3-hour treatment and thus there are no intermediate points of reference (Figure 7). The methoxyl content was determined on samples of pectin extracted from the apricot pulp with boiling water and precipitated with isopropyl alcohol. The data obtained on these precipitates appear in Table 2.

The secondary point where a high degree of rehydration swelling occurred at 26° C. was found when the pectins present had an average methoxyl content of 4.62%. Although this may be mere coincidence, this secondary optimum is at the same methoxyl content of the pectins in the "synthetic" fruit product which showed optimum rehydration capacity at the lower temperature investigated.

At 90°C. the tests showed little value in the variation of methoxyl content of the fruit pectins in rehydration swelling, but retention of water was improved by demethylation to 4.62% CH_3O.

Additional tests were made on apricots which included the use of a smaller proportion of pectin methylesterase and adjustment of the sugar content. No advantages were discovered in these latter experiments.

TABLE 2

Effect of enzymatic demethylation of pectins in apricots upon rehydration swelling of dehydrated apricots

Length of enzyme treatment	Swelling		Water retention		Average CH_3O in pectin
	26°C.	90°C.	26°C.	90°C.	
hours	*ml. per g.*	*ml. per g.*	*g. per g.*	*g. per g.*	%
Control	22.3	19.0	22.6	12.8	11.85
3	19.5	20.1	21.1	10.6	5.80
6	16.7	19.3	19.6	11.1	5.05
12	20.7	18.3	20.1	17.8	4.62
18	19.3	19.0	18.9	17.6	4.40
24	18.7	19.0	19.8	17.8	4.11

SUMMARY

1. Up to 10% of high methoxyl content, pectins in a pectin-cellulose-inert ingredient mixture containing 20% cellulose decreased the rehydration swelling of the cellulose at 26°C. but caused greater swelling at 90°C. than any of the other single ingredients common to fruits tested.

2. A "synthetic" fruit mixture containing cellulose, sugars, salts, acid, and high methoxyl pectin in proportions similar to the average analysis of miscellaneous fruits showed very little rehydration swelling at either 26° or 90°C.

3. Rehydration of a "synthetic" fruit mixture wherein the pectin had been partially deesterified with pectin-methylesterase prior to dehydration showed maximum swelling at 26°C. when the methoxyl content of the pectin was 4.7%.

4. Rehydration of dehydrated apricots whose pectins had been partially demethylated with pectin-methylesterase prior to dehydration showed no value in the deesterification treatment as far as swelling was concerned but retention of solubles was at a maximum at 90°C. when the methoxyl content was 4.7%.

5. No correlation was found between the two types of preparation investigated, namely, the dehydrated simulated fruit preparations and dehydrated apricots.

LITERATURE CITED

1. Baker, G. L., and Murray, W. G. Pectinic acids as related to texture and quality of dehydrated fruit products. *Food Research*, **12**, 129 (1947).
2. Emmett, A. M., and Carré, M. H. A modification of the calcium pectate method for the estimation of pectin. *Biochem. J.*, **20**, 6 (1926).
3. Von Fellenberg, T. Information on the pectins. *Mitt. Lebensm. Hyg.*, **5**, 224 (1914).
4. Winton, A. L., and Winton, K. B. G. *Structure and Composition of Foods*, Volume 2. 1935, John Wiley and Sons, New York, N. Y.
5. Woodmansee, C. W., Baker, G. L., Pollari, V. E., and Murray, W. G. How sugars affect dispersibility of pectins in gel powders. *Food Inds.*, **18**, 356 (1946).

THE ANTIOXIDANT PROPERTIES OF NATURAL SPICES[a,b]

J. R. CHIPAULT, G. R. MIZUNO, J. M. HAWKINS,
AND W. O. LUNDBERG
Hormel Institute, University of Minnesota,
Austin, Minnesota

(Manuscript received June 20, 1951)

Because natural spices are widely used in a variety of food products, it is important to know what effects they have on the keeping qualities of such products. A number of studies have been made on the bactericidal and bacteriostatic properties of spices to evaluate their effectiveness in preventing or retarding spoilage caused by microorganisms. Little attention has been devoted to their ability to retard other types of deterioration, including rancidity caused by the oxidation of fats.

A few scattered observations, however, on the antioxidant properties of spices on fats in certain foods may be found in the literature. Maveety *(5)* patented the use of certain spice fractions for the prevention of rancidity in edible fats. DuBois and Tressler *(3)* reported that black pepper, sage, mace, and ginger retarded development of rancidity in frozen ground pork and beef. Antioxidant properties were attributed to black pepper, red pepper, and sage in stored frozen pork sausage by Atkinson, Cecil, Woodroff, and Shelor *(2)*. Maveety *(6)* found that powdered exhausted cloves increased the stability of lard and of cookies prepared from lard so stabilized. Ramaswamy and Banerjee *(7)* found that the phenolic pigment curcumin, present in turmeric, is responsible for the antioxidant properties of this spice. In a more recent paper, Aggarwal and Sethi *(1)* indicated that chillies, cinnamon, ginger, turmeric, nutmeg, mace, black pepper, cloves, onions, and betel leaf, when heated with ground nut oil to incipient charring at 275° to 280°C.(527° to 536°F.), all retarded the development of peroxides and free fatty acids when the oil was aerated at 100°C.(212°F.). Such treatment might be expected to destroy appreciable quantities of some types of natural antioxidants. However, numerous investigators have shown also that heat treatment of many biological materials, either before or after addition to a fat or oil, produces fat-stabilizing substances. Probably the results reported by Aggarwal and Sethi *(1)* are due in large measure to the development of such stabilizers during heating of the materials at high temperature.

No comprehensive and systematic study of the antioxidant properties of the common natural spices appears to have been attempted hitherto. In the initial phases of this study, therefore, 78 samples representing 32 spices have been examined. First, the stabilizing effect of the ground spices in lard was tested by the active oxygen method at 98.6°C.(210°F.). Also, because the ground spices contain relatively large proportions of antioxygenically inert substances and because the antioxidant properties of the spices may be due to combinations of several primary and syner-

[a] This work was aided by a grant from the American Spice Trade Association.
[b] Hormel Institute publication No. 66.

gistic antioxidants and also prooxidants, at least one sample of each spice was extracted successively with two solvents to separate and concentrate some of the active compounds. The fractions so obtained were also tested in lard by the active oxygen method.

Second, since citric acid, an antioxidant of the synergist type, is used in edible products, and moreover occurs naturally in many food materials to which spices are normally added, the synergistic effect of citric acid on the antioxidant properties of a few spices has also been investigated.

Many antioxidants that are highly effective in a simple fat substrate have been found to be rather ineffective for the prevention of rancidity in baked products in which the fat has been incorporated. The antioxidant is apparently inactivated or destroyed during the preparation and baking of the product. Conversely, at least one compound which is only moderately effective as an antioxidant when used in fats alone has been found to be very effective in baked goods.

In a third phase of this study, therefore, in order to ascertain whether compounds of the latter type are present in spices, pastries of the pie crust type were prepared, using lard with added spices as a shortening, and the stabilities of the baked products determined. Finally, work aimed at the isolation and identification of the antioxidants of a few of the more effective spices has been initiated.

THE ANTIOXIDANT PROPERTIES OF GROUND SPICES AND SPICE FRACTIONS IN LARD

Experimental Methods

Spices that were received in a ground condition were further ground, if necessary, with a mortar and pestle. Whole spices were ground in a small hammer mill. Only materials which passed through a 100-mesh screen were used, except in the cases of two samples of poppyseed which could not be ground to the desired fineness with the available equipment.

It is well known that the inhibiting effectiveness of antioxidants depends not only upon the antioxidant but also upon the nature or composition of the substrate. Differences are evident even with different samples of the same type of fat, and are due, in part at least, to slight differences in the content of antioxidants and of prooxidants naturally present or introduced during the processing of the fat. In order to permit a comparison of the antioxidant properties of the various spices and fractions, therefore, lard from the same batch was used as a substrate in all of the tests described herein. The lard was stored at −20°C.(−4°F.) so that no appreciable changes in stability occurred in the period over which the studies were made.

The ground spices were incorporated in the lard at a concentration of 0.2% and the stabilities of the samples were determined in an apparatus essentially the same as described by King, Roschen, and Irwin *(4)*. The aerating train, however, was modified by adding a condenser to the bottle containing the air-washing solution as recommended by Riemenschneider, Turer, and Speck *(8)*, and by replacing the 3 air-distributing bottles by a single glass manifold that extended over the whole length of the apparatus. Three tubes spaced at appropriate time intervals were used for each sample; all 3 were removed from the apparatus after a rancid odor could be detected in the air effluent of the first tube. The stability of the sample, defined as the time required to reach a peroxide value of 20 meq. per kg., was determined by interpolation from the peroxide values obtained for the 3 tubes. The antioxidant index for each spice was then calculated as the ratio of the stability of the sample containing the spice to the stability of the same lard to which no spice had been added. The stability of the lard itself was 6.5 hours.

Only one sample each of allspice, caraway, cloves, marjoram, savory, and thyme was available for testing. In all other cases, two or more samples of each spice were examined and these were selected to vary as much as possible with respect to origin and supplier.

Inasmuch as many, if not most, organic compounds exhibit a more or less distinct preference in their affinity for either polar or nonpolar types of solvents, two fractions of each spice sample were obtained by successive treatment of the sample with a nonpolar solvent, petroleum ether (Skellysolve F), and a polar solvent, ethyl alcohol (95%). Then 25 g. of the ground spice were weighed into a 250-ml. centrifuge bottle and enough petroleum ether added to wet the spice thoroughly. An additional 50 ml. of solvent was added and the mixture thoroughly shaken on a mechanical shaker for 30 minutes. After a brief centrifugation, the supernatant liquor was decanted through a filter paper into a 250-ml. volumetric flask. The residual material was treated 5 more times in the same manner using 50-ml. portions of petroleum ether. To determine the amount of material dissolved, the combined supernatants were adjusted to a volume of 250 ml. and an aliquot removed and freed of solvent at room temperature.

After the petroleum ether had evaporated from the original spice residue, a second fraction was similarly obtained by treating the residue with 6 successive 50-ml. portions of ethyl alcohol. The combined filtered alcoholic extracts were also adjusted to 250 ml. and an aliquot removed for a determination of the amount of dissolved material.

The test lard samples were prepared by adding 2 ml. of a solvent fraction to 100 g. of lard, followed by removal of the solvent by evaporation. Since 2 ml. of the solvent fraction contained soluble material from 0.2 g. of the whole spice, the concentration of any solvent fraction was equivalent for comparative purposes with a 0.2% concentration of the ground spice.

Results

The results of the stability measurements for the spices and equivalent solvent fractions in terms of antioxidant indexes are given in Table 1, columns 2, 3, and 7. In most cases there was little difference between the antioxidant indexes of various samples of the same spice, indicating that in general the antioxidant activities of spices tend to be relatively independent of their origin. To conserve space, therefore, not all of the samples tested are included in Table 1. One representative sample of each spice is listed, except that for some of the spices which proved to be most strongly antioxygenic, two samples have been included in the table. Similarly, not all spice samples were used in the preparation of solvent fractions. In most cases, one representative sample was selected.

The table contains additional information concerning the solvent-soluble fractions. Columns 4 and 8 give the actual concentrations in lard of the soluble materials from the petroleum ether-soluble and alcohol-soluble fractions, respectively. Columns 5 and 9 contain theoretical antioxidant indexes for the petroleum ether-soluble and alcohol-soluble fractions at 0.2% actual concentration in lard. The following formula was used:

$$\frac{(\text{Observed antioxidant index} - 1) \times 0.2}{\text{Actual concentration}} + 1.$$

In this calculation the assumption is made that the increase in stability of the lard is proportional to the concentration of the fraction employed. Although the assumption is generally valid at low concentrations, the values should be regarded only as approximations for some, and possibly most of the fractions at concentrations of 0.2%. Nevertheless, the calculated indexes in columns 5 and 9 are useful in giving approximate indications

TABLE 1

Antioxidant properties of ground spices and of petroleum ether and alcohol-soluble fractions [a]

Spice	Ground spice	Petroleum ether-soluble fraction				Alcohol-soluble fraction			
	Antioxidant index	Antioxidant index	Actual concentration in lard	Theoretical antioxidant index at 0.2%	Concentration factor	Antioxidant index	Actual concentration in lard	Theoretical antioxidant index at 0.2%	Concentration factor
			%				%		
Allspice	1.8	1.1	0.012	2.7	2.1	1.0	0.016	1.0	0
Aniseed	1.9	1.1	0.049	1.4	0.5	1.2	0.014	3.9	3.2
Basil leaves	1.2	1.0	0.008	1.0	0	0.8	0.012	....	....
Bay leaves	2.1	1.3	0.019	4.2	2.9	1.3	0.036	2.7	1.5
Cardamom	1.3	0.8	0.008	....	....	1.1	0.007	3.9	9.5
Caraway	1.8	0.9	0.043	....	....	1.2	0.011	4.6	4.6
Cassia	1.4	1.0	0.007	1.0	0	1.3	0.030	4.0	7.5
Celery seed	1.2	0.9	0.058	....	....	1.1	0.011	2.9	9.1
Chilli	1.5	1.3	0.027	3.2	4.4	1.4	0.049	2.6	3.2
Cinnamon	1.3	0.9	0.004	....	....	1.4	0.017	5.7	15.7
Cloves	1.8	1.4	0.034	3.4	2.9	1.7	0.027	6.2	6.5
Coriander	1.3	0.9	0.034	....	....	1.0	0.010	1.0	0
Cumin	1.3	1.1	0.017	2.2	3.9	1.2	0.018	3.2	7.4
Dill	1.3	0.8	0.010	....	....	1.0	0.012	1.0	0
Fennel	1.3	0.9	0.017	....	....	1.1	0.012	2.7	5.6
Foenugreek	1.6	0.9	0.005	....	....	1.2	0.013	4.1	5.1
Ginger	1.8	1.0	0.006	1.0	0	1.5	0.004	26.0	31.3
Mace	2.6	1.1	0.056	1.4	0.2	1.8	0.010	17.0	10.0
Marjoram	2.2	1.0	0.010	1.0	0	1.3	0.015	5.0	3.3
Mustard	2.0	1.1	0.068	1.3	0.3	1.4	0.014	6.7	5.7
Nutmeg	3.1	1.0	0.073	1.0	0	2.2	0.011	22.8	10.4
Oregano A	3.8	1.4	0.011	8.3	2.6	2.7	0.025	14.6	4.9
Oregano B	3.3	1.8	0.007	23.9	6.9	2.6	0.025	13.8	5.6
Paprika	2.5	1.2	0.016	3.5	1.7	1.4	0.028	3.9	1.9
Pepper, black	1.4	1.0	0.007	1.0	0	0.9	0.011	....	....
Pepper, red	1.5	1.1	0.010	3.0	4.0	1.3	0.023	3.6	5.2
Pepper, white	1.2	1.0	0.005	1.0	0	0.9	0.012	....	....
Poppy seed	1.2	0.7	0.078	....	....	1.2	0.013	4.1	15.4
Rosemary A	17.6	2.2	0.010	25.0	1.4	5.5	0.034	27.5	1.6
Rosemary B	17.6	2.5	0.013	24.1	1.4	5.2	0.030	29.0	1.7
Sage A	14.2	2.2	0.014	18.1	1.3	5.4	0.027	33.6	2.5
Sage B	16.5	2.2	0.010	25.0	1.5	5.2	0.022	39.2	2.5
Savory	1.6	1.3	0.005	13.0	20.0	1.3	0.012	6.0	8.3
Thyme	3.0	1.5	0.008	13.5	6.3	3.0	0.018	23.2	11.1
Turmeric A	2.9	1.0	0.008	1.0	0	2.0	0.018	12.1	5.8
Turmeric B	2.0	1.0	0.007	1.0	0	2.1	0.017	13.2	12.2

[a] Active oxygen method at 98.6°C., employing as substrate prime steam lard with a stability of 6.5 hours.

of the comparative antioxidant potencies of the solvent fractions from different spices.

Columns 6 and 10 contain a calculated concentration factor obtained by the following equation:

$$\frac{\text{Observed antioxidant index of solvent fraction} - 1}{\text{Observed antioxidant index of ground spice} - 1} \times \frac{0.2}{\text{Actual concentration of solvent fraction}}$$

The values so obtained are useful in indicating the multiplication of the overall antioxidant potency of the ground spice that was achieved by solvent fractionation. They have no theoretical significance and cannot be relied upon as quantitative measures of the extent to which any individual compounds have been concentrated; in conjunction with the other data they merely serve as useful guides toward a further concentration and separation of antioxidant components of the spices.

Insofar as the antioxidant indexes of spices are concerned (column 2, Table 1), of the 78 samples tested, only one (ginger) proved to be consistently prooxygenic. Three other samples of ginger, however, possessed slight and approximately equal antioxidant activities, and the data for one of these is given in the table. In all other spices, the antioxidant activity varied from slight to very pronounced. Rosemary and sage were particularly potent.

Columns 3 and 7 reveal that, at concentrations equivalent to 0.2% of the ground spices, the solvent fractions, with four exceptions, did not exhibit antioxidant indexes as high as those of the ground spices. In the cases of cinnamon, poppyseed, thyme, and turmeric (sample B), the alcohol-soluble fractions were equal to or slightly better than the ground spice while the petroleum ether-soluble fractions were inactive or prooxygenic, except for thyme. With 12 spices, one of the fractions acted as a prooxidant. In the cases of black pepper and white pepper, both fractions were either prooxygenic or inactive even though the ground spices were slightly antioxygenic.

From these results it must be concluded that in general the ground spices exert a greater antioxygenic effect than equivalent concentrations of the solvent-soluble fractions obtained from them. In those few cases which do not conform to this rule, it is probable that prooxidants account for the lower effectiveness of the ground spices.

The values in columns 5 and 9, Table 1, are calculated approximations of the antioxidant indexes of the solid fractions when employed at 0.2% concentrations. These values, therefore, afford a basis for comparing the antioxygenic activity of the solvent-soluble fractions at equal concentrations. It will be noted that again sage and rosemary were prominent in providing the most potent materials and this was true of both the petroleum ether-soluble and alcohol-soluble fractions. However, it is also to be noted that oregano, savory, and thyme all yielded potent petroleum ether-soluble fractions and that ginger, mace, nutmeg, oregano, thyme, and turmeric also yielded potent alcohol-soluble fractions. In those cases where potent solvent fractions were obtained from spices which do not exhibit a marked antioxidant activity in the form of ground spice, the explanation in

most instances is that the antioxidants or antioxidant combinations found in the solvent-soluble fractions are not present in appreciable proportions in the original ground spice.

Columns 6 and 10, Table 1, indicate the extent to which the antioxidant properties of the ground spices were concentrated in the solid fractions. A quite appreciable concentration of the antioxidant effectiveness was achieved in the petroleum ether-soluble fractions of chilli, cumin, oregano, red pepper, savory, and thyme, and in the alcohol-soluble fractions of cardamom, caraway, cassia, celery seed, cinnamon, cloves, cumin, fennel, foenugreek, ginger, mace, mustard, nutmeg, red pepper, poppy seed, savory, thyme, and turmeric.

SYNERGISTIC COMBINATIONS OF CITRIC ACID AND ALCOHOL-SOLUBLE FRACTIONS OF SOME SPICES

The alcohol-soluble fractions of nutmeg, oregano, rosemary, and sage, obtained as previously described, were incorporated in lard samples at concentrations equivalent to 0.2% of the ground spice, and citric acid was also added to each sample to give concentrations of 0.1%. The stabilities of the samples were then measured by the active oxygen method at 98.6°C. (210°F.). The results are given in Table 2.

TABLE 2

Synergism of citric acid with the alcohol-soluble fractions of spices [4]

Spice sample	Actual concentration of alcohol-soluble fraction of spice	Antioxidant index
	%	
Control plus citric acid	.....	1.57
Control plus nutmeg	0.011	2.24
Control plus nutmeg plus citric acid	0.011	3.12
Control plus oregano B	0.024	2.61
Control plus oregano B plus citric acid	0.024	3.28
Control plus rosemary A	0.030	5.20
Control plus rosemary A plus citric acid	0.030	9.19
Control plus sage B	0.022	5.20
Control plus sage B plus citric acid	0.022	7.20

[4] Citric acid concentration, 0.1%, alcohol-soluble fraction concentration equivalent to 0.2% of ground spice. Stability of control sample of lard was 5½ hours.

By definition a synergistic effect exists if the following is true:

$$(\text{Antioxidant index of the combination of antioxidants} - 1) > \Sigma\,(\text{Antioxidant index of each antioxidant alone} - 1)$$

Examination of the antioxidant indexes reveals that virtually no synergistic effect was obtained with oregano, but that some effect was found with nutmeg and sage, and that a rather pronounced effect was obtained with rosemary. These results suggest that further studies of this character may be useful in characterizing the nature of the antioxygenic properties of spices. Thus the occurrence of little or no synergistic effect with citric

acid suggests that probably a significant proportion of the natural antioxidants in the spice are of the acid or synergist type. On the other hand, the occurrence of a marked synergistic effect suggests that probably the antioxidants are largely of the primary type (phenols, aromatic amines, etc.) and that significant amounts of synergist types are not present.

THE ANTIOXIDANT EFFECTS OF GROUND SPICES IN PIE CRUSTS

Experimental Methods

In order to insure uniformity during baking, it was necessary to devise an oven in which all of the samples would be submitted, as nearly as possible, to the same conditions of temperature and air circulation. A well-insulated constant temperature oven was therefore equipped with a recirculating blower and a slowly rotating turntable on which the samples were placed during baking.

The pie crust samples were prepared and handled exclusively with glass utensils. The procedure was as follows: Flour (commercial enriched) and salt (noniodized kitchen salt) were first thoroughly blended in a dry state in proportions of 100 to 3.65 by weight. Ground spice was dispersed into the melted lard at a concentration of 0.2%. A 5.7-g. portion of the flour-salt mixture was weighed into a 50-ml. beaker, and 3.5 g. of the melted lard was added and mixed in with a glass stirring rod for 1.5 minutes. Water (0.6 ml.) was then added and mixed with the other ingredients for 2 minutes. The dough was transferred to 2 x 3 inch glass plates and rolled out with a glass roller supported by glass plates on each end to a uniform thickness of about ⅛ inch. The thin ragged edges were pressed back slightly toward the center of the slab during the rolling operation to prevent the development of thin areas. Care was taken also to press air bubbles out of the dough. The glass plates supporting the samples were then placed on a metal sheet and the latter placed on the turntable in the oven. The samples were kept in the oven for 13 minutes at a setting of 194°C.(381°F.). Doughs representing 6 spice samples and a control were prepared and baked in duplicate at one time.

After baking, the crusts were removed from the oven and allowed to cool briefly. The duplicates were then combined, broken up and transferred to a 4-oz. wide-mouthed bottle, loosely covered with an inverted beaker. These were placed in an incubating oven at 63°C.(145.5°F.) and their odor examined by two or more observers at least twice daily. In addition, 1-g. samples of pie crusts were removed from time to time and analyzed for peroxide content.

The procedure in the peroxide determination was as follows: One gram of pie crust was weighed into a 125-ml. Erlenmeyer flask and 8 ml. of a mixture of chloroform and acetic acid (2:3 by volume) were added. The crumbs were thoroughly broken up by pressing with a glass rod flattened at one end, and the mixture was well agitated for a few minutes and the liquid decanted into a filter. Two additional 8-ml. extracts of the residue were made in the same manner and combined with the first extract. Saturated aqueous potassium iodide (0.5 ml.) was added to the chloroform-acetic acid filtrate. After 2 minutes, 30 ml. of water was added and the free iodine titrated with 0.01 *N*. thiosulfate, using a starch indicator.

Experience showed that with the pie crust formula and the experimental conditions used, the chloroform-acetic acid solvent consistently extracted 0.32 g. of fat from 1 g. of pie crust. Once this was established, measurements of the weight of the extracted fat were no longer necessary; instead the weight of extracted fat was calculated from the weight of pie crust used. The peroxide values were calculated as meq. of peroxidic oxygen per 1000 g. of fat.

In general, the incidence of rancidity as detected by odor examination agreed very well with the induction period determined by peroxide analysis. With few exceptions, samples remained fresh below a peroxide value of 17.0 and few samples with a peroxide value of more than 25 failed to smell rancid. In a few samples, the spice odor itself was strong enough to interfere with the detection of incipient rancidity. However, even in these

samples, once the point of incipient rancidity was reached, as indicated by the peroxide measurements, the peroxides (and hence their decomposition products) increased so rapidly that a rancid odor soon became evident.

In some cases the work schedule did not allow odor examination of the samples at sufficiently frequent intervals, for example, during the overnight periods. Some samples with peroxide values of 10 to 20 at the end of the working day did not smell rancid, but by the next morning these samples would often be extremely rancid and have peroxide values of 100 or more. For this reason the odor examinations have been used simply to corroborate the chemical measurements, and the recorded stabilities of the samples are based on peroxide analysis. For each sample, a curve relating the peroxide values to storage time was drawn. The time necessary for the fat in a sample to reach a peroxide value of 20 meq. per kg. was determined from the curve and taken as the stability of the sample.

TABLE 3

Antioxidant activity of ground spices in pie crusts

Spice	Antioxidant Index	Spice	Antioxidant Index
Allspice	1.13	Marjoram	1.09
Aniseed	1.25	Mustard	1.40
Basil leaves	1.00	Nutmeg	1.44
Bay leaves	1.16	Oregano A	2.74
Cardamom	0.90	Oregano B	3.22
Caraway	1.13	Paprika	1.05
Cassia	0.95	Pepper, black	1.09
Celery seed	1.01	Pepper, red	0.98
Chilli	1.19	Pepper, white	1.06
Cinnamon	0.92	Poppy seed	0.99
Cloves	1.32	Rosemary A	4.10
Coriander	0.80	Rosemary B	4.48
Cumin	0.90	Sage A	2.74
Dill	0.76	Sage B	2.95
Fennel	0.71	Savory	1.18
Foenugreek	0.84	Thyme	1.94
Ginger	1.09	Turmeric A	1.28
Mace	1.42		

Table 3 gives the antioxidant indexes in pie crusts for 35 spice samples. Each value is the average of two or more separate bakings. Because of many factors, some of which cannot be closely controlled, it is not possible to produce measurements of antioxidant effectiveness in baked products with a high degree of accuracy. The average deviation of individual antioxidant indexes from the mean values given in the table was 8.2% (range 0.5 to 25.9%) for the entire series of baking experiments. The values are therefore accurate enough to indicate definitely any important differences between spices.

The stabilities of pie crust samples containing dill, fennel, foenugreek, cardamom, cumin, and coriander have consistently been less than those of the corresponding controls. For the most part, the other spice samples are either inactive or very slightly antioxygenic. Oregano, rosemary, sage and thyme improved the stability of the baked products quite significantly,

although their stabilizing effect was not as great as in lard alone. A reduced effectiveness has similarly been found to occur with most well known antioxidants and has usually been attributed to a destruction of the antioxidants during the preparation of the baked product.

The three most effective spices, however, show some notable differences. While rosemary and sage were approximately equal in their abilities to retard rancidity in lard, rosemary appears definitely superior to sage when used in pie crust. Oregano is at least equal to sage in pie crust but much inferior in lard alone. It appears that the antioxidants of oregano either are not so easily destroyed during the baking process, or that their destruction is compensated to a greater extent than in sage by the development of other antioxidants from materials in the spice as a result of the heat treatment.

ISOLATION OF ANTIOXIDANTS FROM SPICES

Efforts have been directed toward the isolation of individual antioxidants in sage and rosemary, and the results of such studies will be reported in detail in a later publication. However, the preliminary fractionations demonstrated the following:

1. Sage contains at least two potent antioxidants having quite different physical and chemical properties.
2. The predominant antioxidants in sage and rosemary are soluble in aqueous alkali, suggesting an acidic (or phenolic) character.
3. Fractions containing the predominant antioxidants of sage and rosemary do not contain the characteristic odor components of these spices; conversely, fractions containing the odor components exhibit little antioxidant activity.

Summary

The antioxidant properties of samples of 32 spices have been examined. With one exception in the 78 samples examined (one sample of ginger) the ground spices in all cases exhibited an antioxidant effect on prime steam lard when tested by the active oxygen method at 98.6°C.(210°F.). Rosemary and sage exhibited particularly pronounced antioxidant effects.

Petroleum ether-soluble and alcohol-soluble fractions of these spices, with few exceptions, did not exhibit antioxygenic activities as marked as those of ground spices when employed at equivalent concentrations. However, some of the solvent fractions were decidedly active, although in some cases the highly potent antioxidants appeared to be present in the ground spices in quite low concentrations.

Citric acid exerts a synergistic effect on the antioxidant properties of some spice fractions and no effect on others.

In general, the antioxidant activity of the spices in pie crust was considerably less than in lard, due probably to a partial destruction of antioxidants during baking. The antioxidants of some of these spices appeared to be less susceptible to destruction by heating than those of other spices.

Acknowledgment

The authors are indebted to the members of the Research Committee of the American Spice Trade Association and the companies they represent for generously supplying all of the samples of spices that have been used in this investigation.

LITERATURE CITED

1. AGGARWAL, J. S., AND SETHI, S. C. Stabilization of edible fats by condiments or spices. *Nature*, **166**, 518 (1950).
2. ATKINSON, IDA, CECIL, S. R., WOODROFF, J. G., AND SHELOR, ETHYL. Extending keeping quality of frozen pork sausage. *Food Ind.*, **19**, 1198 (1947).
3. DUBOIS, C. W., AND TRESSLER, D. K. Seasonings, their effect on maintenance of quality in storage of frozen ground pork and beef. *Proc. Inst. Food Technol.*, 202 (1943).
4. KING, A. E., ROSCHEN, H. L., AND IRWIN, W. H. An accelerated stability test with the peroxide value as an index. *Oil and Soap*, **10**, 105 (1933).
5. MAVEETY, D. J. U. S. Patent 2,124,706 (July 26, 1938).
6. MAVEETY, D. J. Correlation of keeping properties of shortenings with keeping quality of biscuits. *Oil and Soap*, **23**, 25 (1946).
7. RAMASWAMY, T. S., AND BANERJEE, B. N. Vegetable dyes as antioxidants for vegetable oils. *Ann. Biochem. and Exptl. Med. (India)*, **8**, 55 (1948).
8. RIEMENSCHNEIDER, R. W., TURER, J. AND SPECK, R. M. Modifications of the Swift stability test. *Oil and Soap*, **20**, 169 (1943).

THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF PROCESSED MEATS[a]

B. S. SCHWEIGERT, BARBARA A. BENNETT, MONA MARQUETTE, HAROLD E. SCHEID, AND B. H. McBRIDE

Division of Biochemistry and Nutrition, American Meat Institute Foundation and Department of Biochemistry, University of Chicago, Chicago, Illinois

(Manuscript received June 20, 1951)

Extensive studies have been conducted on the vitamin content of uncooked and cooked fresh meats; however, the data available on the vitamin content of various processed meats are limited. The ready-to-eat meats classified as sausages, for example, constitute approximately 10% of the total meat production of government-inspected plants *(2)*. The purpose of the present studies was to determine the proximate composition and thiamine, riboflavin, and niacin content of certain processed meats obtained during 1949 and also in 1950. The samples selected for this study included sausage items, canned meat products, and cured meats.

EXPERIMENTAL METHODS

The meat samples were purchased from a retail store that had a high turnover and large meat trade. Two or more pounds of each type of meat were obtained and the sausage and luncheon meat samples were taken from uncut pieces of meat that had not been in the showcase. For the canned meats packed in less than 1-pound cans the entire contents of 4 or 5 cans were used and pooled. All samples were ground and thoroughly mixed to assure homogeneity. They were then stored at 23°F.(5°C.) prior to analysis. These samples were analyzed for proximate composition, thiamine, riboflavin, and niacin. Thiamine was determined fluorometrically, riboflavin both fluorometrically and microbiologically, and niacin microbiologically with the use of methods similar to those described by the Association of Vitamin Chemists *(1)*.

The proximate analyses and vitamin content of the samples obtained in 1949 are summarized in Table 1 and for 1950 in Table 2.

RESULTS AND DISCUSSION

The results show that all of the meat products except head cheese, potted meat, and bacon were excellent sources of niacin. Bacon and head cheese were low in riboflavin. When the vitamin content of the samples are compared on an equal protein basis bacon compares favorably with the other samples in vitamin content.

Thiamine was low in all products that did not contain pork. For the pork products, the thiamine content was variable, which undoubtedly reflected the severity of the processing methods, the amount of pork used in the products, the variation in thiamine content of different muscles and the thiamine nutrition of the animals before slaughter *(2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14)*. The values for the pork products studied varied from 0.15 to 0.50 mg. per 100 g., while values from 0.6 to 1.5 have been observed for uncooked pork loin and ham samples. Although the values are not strictly

[a] The proximate analyses were done by the Service Laboratory of the American Meat Institute Foundation. Journal paper No. 39, American Meat Institute Foundation.

TABLE 1

B vitamin content of processed meats obtained in 1949

Sample description[b]	Moisture	Ether extract	Protein	Thiamine	Riboflavin	Niacin
	%	%	%	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
Sausage items (cured)						
Thuringer (beef and pork)	48.50	25.22	17.72	0.117	0.262	3.81
Cooked salami (beef and pork)	54.45	21.95	17.10	0.148	0.214	3.56
Kolbase, Polish (pork and pork cheeks)	50.80	32.40	13.51	0.287	0.211	2.93
Bologna, large (beef and pork)	59.95	21.15	12.95	0.082	0.270	2.87
Sausage, country style, smoked (pork)	50.60	32.30	14.98	0.269	0.230	3.49
Sausage, links, smoked (beef, veal, and pork)	46.85	33.62	15.35	0.213	0.180	3.02
Dutch loaf (beef and pork)	59.70	16.12	14.99	0.269	0.177	2.99
Head cheese (pork snouts, head meats, skins)	54.65	25.82	15.07	0.026	0.110	0.46
Braunschweiger (pork and pork livers)	45.80	30.80	12.53	0.103	1.57	7.64
Canned products						
Veal loaf (veal and pork)	60.08	15.42	17.22	0.027	0.212	3.35
Luncheon meat, spiced (cured pork)	49.70	31.60	12.18	0.268	0.155	2.21
Ham, chopped (cured)	62.00	16.95	16.77	0.314	0.196	3.16
Pork, chopped (cured shoulder and ham)	54.35	28.40	13.77	0.222	0.197	2.41
Pork, chopped (cured shoulder and ham)	54.80	24.35	14.63	0.266	0.225	2.49
Pork and beef (cured)	57.45	22.95	14.29	0.217	0.205	2.62
Vienna sausage (beef and pork)	68.00	16.82	12.39	0.038	0.164	2.73
Potted meat (beef, tripe, heart)	65.20	16.32	16.08	0.027	0.225	1.22
Beef, chopped (cured)	63.35	15.82	17.05	0.014	0.163	4.96
Beef, diced	74.95	3.03	20.78	0.012	0.228	4.77
Veal, diced	76.70	1.25	21.00	0.029	0.305	6.73
Pork, diced	71.60	5.04	20.96	0.253	0.250	4.75
Beef liver, diced	70.50	3.74	21.76	0.072	3.11	13.83
Lamb, strained	76.60	5.70	16.89	0.022	0.208	3.89
Miscellaneous						
Ham, boiled	52.60	27.55	14.26	0.385	0.139	1.75
Beef tongue, smoked	48.90	28.80	17.21	0.042	0.211	3.00
Bacon	18.78	69.48	7.76	0.220	0.110	1.30

[b] The major ingredients as listed on the label for each sample are indicated.

comparable since the original thiamine values before processing were not known, the data would suggest that considerable destruction of thiamine occurred during the processing of the canned pork samples, and the retention of thiamine was somewhat less than during the cooking of fresh pork by standard procedures *(8)*.

Although the values found for the same products for the two years of sampling were quite uniform, particularly in the case of niacin, the values were quite different in certain cases. The variation in the values for boiled ham, for example, may be accounted for, in part, by the differences in proximate composition. The sample used in 1949 contained 14.3% protein while the 1950 sample contained 19.6% protein.

TABLE 2

B vitamin content of processed meats obtained in 1950

Sample description[c]	Moisture	Ether extract	Protein	Thiamine	Riboflavin	Niacin
	%	%	%	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
Sausage items (cured)						
Thuringer (beef and pork)	50.27	23.29	16.03	0.116	0.205	4.55
Cooked salami No. 1 (beef and pork)	50.68	19.94	17.28	0.211	0.195	3.95
Cooked salami No. 2 (beef and pork)	31.90	36.21	22.38	0.409	0.211	5.84
Kolbase, Polish (pork and pork cheeks)	50.56	25.11	13.54	0.392	0.163	3.30
Bologna, large (beef and pork)	58.90	17.78	13.82	0.137	0.181	2.46
Sausage, links, smoked (beef, veal, and pork)	45.35	30.88	13.62	0.230	0.203	3.15
Dutch loaf (beef and pork)	57.00	12.67	13.62	0.348	0.164	3.31
Head cheese (pork snouts, head meat, and skins)	63.43	12.27	14.05	0.032	0.083	0.60
Braunschweiger (pork and pork liver)	47.12	32.64	13.38	0.152	1.25	8.52
Canned products						
Veal loaf (veal and pork)	61.28	15.34	17.38	0.025	0.161	3.77
Luncheon meat, spiced (pork)	50.84	27.30	12.93	0.392	0.157	2.91
Ham, chopped (cured)	58.69	16.45	16.49	0.333	0.152	3.55
Ham, deviled (chopped ham and ham fat)	46.01	37.81	12.57	0.172	0.109	2.10
Pork, cured (shoulder and ham)	55.75	22.04	14.11	0.301	0.147	3.02
Vienna sausage (pork, beef, and hearts)	65.21	15.58	13.62	0.037	0.144	2.60
Beef, chopped (cured)	62.30	16.61	16.03	0.019	0.137	4.85
Corned beef	57.52	13.70	19.46	0.035	0.153	2.74
Hamburgers	59.21	19.62	17.14	0.034	0.144	3.75
Frankfurters (beef and pork)	63.07	17.65	8.74	0.020	0.090	2.07
Pork sausage	46.58	31.46	13.96	0.257	0.158	3.35
Pork, diced	71.93	6.32	21.22	0.153	0.250	4.57
Beef, diced	75.52	3.17	20.89	0.033	0.175	5.20
Beef liver, diced	68.89	4.25	21.68	0.028	2.13	14.05
Veal, strained	82.36	3.33	13.92	0.013	0.100	2.45
Miscellaneous items						
Ham, boiled	59.37	13.50	19.61	0.501	0.161	3.46
Pork tongue, jellied	47.76	16.24	24.71	0.061	0.322	2.70
Bacon	18.67	72.03	8.67	0.204	0.066	1.82

[c] See footnote [b], Table 1.

The thiamine, riboflavin, and niacin contents of some of the same or similar samples have been reported *(2, 9, 15)*. In view of the wide variations in thiamine content of the original samples, and of processing conditions, it is of interest that the values for thiamine are in rather good agreement.

SUMMARY

The proximate composition, thiamine, riboflavin, and niacin content of a series of processed meats (sausage items, canned meat products, etc.) were determined for samples procured in 1949 and 1950. Except for head cheese, potted meat, and bacon, all samples were excellent sources of niacin. Bacon and head cheese were low in riboflavin content when expressed as mg. per 100 g. of the undried products. On an equal protein basis bacon

compares favorably with the other processed products in vitamin content.

The thiamine content was low in all samples that did not contain pork. The processed pork samples varied in thiamine content from 0.15 to 0.50 mg. per 100 g.

LITERATURE CITED

1. Association of Vitamin Chemists. *Methods of Vitamin Assay.* 1947, Interscience Publishers, Inc., New York City.
2. Beuk, J. F., Fried, J. F., and Rice, E. E. Nutritive values of sausage and other table-ready meats as affected by processing. *Food Research*, **15**, 302 (1950).
3. Cover, S., Dilsaver, E. M., and Hays, R. M. Retention of B vitamins after large-scale cooking of meat. I. Suitability of left and right muscles for retention studies. *Food Research*, **13**, 472 (1948).
4. Cover, S., Dilsaver, E. M., Hays, R. M., and Smith, W. H. Retention of B vitamins after large-scale cooking of meat. II. Roasting by two methods. *J. Am. Dietet. Assoc.*, **25**, 949 (1949).
5. Greenwood, D. A., Beadle, B. W., and Kraybill, H. R. Stability of thiamine to heat. II. Effect of meat-curing ingredients in aqueous solutions and in meat. *J. Biol. Chem.*, **149**, 349 (1943).
6. Heinemann, W. W., Ensminger, M. E., Cunha, T. J., and McCulloch, E. C. The relation of the amount of thiamine in the ration of the hog to the thiamine and riboflavin content of the tissue. *J. Nutrition*, **31**, 107 (1946).
7. Jackson, S. H., Crook, A., Malone, V., and Drake, T. G. H. The retention of thiamine, riboflavin and niacin in cooking pork and in processing bacon. *J. Nutrition*, **29**, 391 (1945).
8. McIntire, J. M., Schweigert, B. S., Henderson, L. M., and Elvehjem, C. A. The retention of vitamins in meat during cooking. *J. Nutrition*, **25**, 143 (1943).
9. McIntire, J. M., Schweigert, B. S., Herbst, E. J., and Elvehjem, C. A. Vitamin content of variety meats. *J. Nutrition*, **28**, 35 (1944).
10. Miller, R. C., Pence, J. W., Dutcher, R. A., Ziegler, P. T., and McCarthy, M. A. The influence of the thiamine content of the pig on the thiamine content of pork with observations on the riboflavin content of pork. *J. Nutrition*, **26**, 261 (1943).
11. Noble, I., Gordon, J., and Catterson, L. Thiamine and riboflavin retention in pork sausage. *J. Am. Dietet. Assoc.*, **25**, 50 (1949).
12. Rice, E. E., and Beuk, J. F. Reaction rates for decomposition of thiamine in pork at various cooking temperatures. *Food Research*, **10**, 99 (1945).
13. Rice, E. E., Daly, M. E., Beuk, J. F., and Robinson, H. E. The distribution and comparative content of certain B-complex vitamins in pork muscular tissues. *Arch. Biochem.*, **7**, 239 (1945).
14. Schweigert, B. S., McIntire, J. M., and Elvehjem, C. A. The retention of vitamins in pork hams during curing. *J. Nutrition*, **27**, 419 (1944).
15. Watt, B. K., and Merrill, A. L. *Composition of Foods.* 1950. U. S. D. A. Agric. Handbook No. 8, Washington, D. C.

INFLUENCE OF CARBOHYDRATE AND NITROGEN SOURCES UPON LIPID PRODUCTION BY CERTAIN YEASTS

S. S. HUSAIN[a] AND M. M. HARDIN
Department of Bacteriology, University of Kentucky, Lexington, Kentucky

(Manuscript received July 25, 1951)

The origin and fate of fatty material in microorganisms has been a subject of investigation for many years. Numerous microorganisms have been shown to accumulate lipid from non-lipid materials in varying degrees. Of special interest are certain lipid producing yeasts, which have been studied from the viewpoint of large scale production of lipids. Aside from *Endomyces vernalis*, which has been most widely used in the past, good lipid yields from several other types of yeasts have been reported. Rippel *(7)* reported on the lipid contents of *Torulopsis pulcherima*, an unidentified strain of red yeast, and *Nectaromyces reukauffi*. He found *Nectaromyces reukauffi* to be the most promising as it produced 15 g. of lipid from 100 g. of sugar in 23 days. Nilsson, Enebo, Lundin, and Myrbäck *(5)* reported that of several yeasts tested *Rhodotorula glutinis* gave the highest lipid yield. When grown in submerged cultures, it produced 14.1% lipid within 2-3 days. Kleinzeller *(2)* obtained lipid yields from 18.6% to 43% on a dry basis with *Torulopsis lipofera*, a soil yeast. This yeast grew well and readily converted carbohydrate to lipid on a medium containing 0.05% yeast extract, glucose, ammonia, and mineral salts. A molasses medium containing potassium, magnesium, and manganese salts, adjusted to a pH of 5.5-6.0, was also favorable for growth and lipid production. The optimum temperature was 20°-25°C. Starkey *(9)* isolated from soil a yeast which produced lipid yields of 48 to 65%. Recently much attention has been given to *Rhodotorula gracilis* as a high lipid producer, as indicated in the excellent review on fat synthesis by microorganisms, published by Lundin *(3)*.

The present investigation was undertaken in the light of the fact that, although reports concerning different physical and chemical factors that influence lipid synthesis by yeasts have appeared from time to time, information on the role of specific carbohydrates and sources of nitrogen on the overall yield of lipids per cell is still of fragmentary nature. A study of the effects of various carbohydrates and nitrogenous substances on the cell yields and on the lipid yields of a few selected strains of yeasts was undertaken in view of the lack of systematic studies in this field.

EXPERIMENTAL PROCEDURE

Cultures. One culture each of *Candida arborea*, *Torula utilis*, and *Rhodotorula gracilis* has been used. The culture of *Rhodotorula gracilis* was obtained through the courtesy of Dr. S. C. Pan, of the Research Laboratories, Joseph E. Seagram and Sons, Inc., Louisville, Ky. The other two yeasts were obtained from the U. S. Forest Prod-

[a] Present address: Department of Bacteriology and Parasitology, University of Chicago, Chicago, Illinois.

ucts Laboratory, Madison, Wis. These and other yeasts have been used in Germany for the production of fodder yeast.[b]

Strictly speaking, as clearly established by Skinner, Emmons, and Tsuchiya *(8)* members of the genus *Candida* are yeast-like fungi rather than typical yeasts, since they form a true mycelium under certain conditions. Also, the generic name *Torula* is not valid; instead, *Cryptococcus* is the correct name in accordance with the International Code of Nomenclature. However, in this paper the organisms used will be spoken of as yeasts and be referred to by the names applied by their donors.

All cultures were maintained on Sabouraud's agar slants (1% glucose, 1% peptone). They were transferred every 2 weeks. After 24 hours of incubation at room temperature, they were kept in the refrigerator.

General procedure. Cultures were grown in 500-ml. Erlenmeyer flasks under continuous aeration for 72 hours at room temperature in 200 ml. of basal medium containing various added nitrogen sources and sugars. Determinations were made of the dry weight of yeast produced and of the lipid content of the yeast. From the results of these determinations the dry weight of yeast per 100 g. of sugar, on the basis of the initial sugar content, and grams of lipid per 100 g. dry weight of yeast were calculated. All experiments were carried out in duplicate. Results given in tables are averages of such duplicate experiments.

The following modification of a synthetic medium described by Starkey *(9)* was used.

Glucose (or other carbohydrate being tested)	22.2 g.
Yeast extract	0.1 g.
K_2HPO_4	0.8 g.
$MgSO_4 \cdot 7\ H_2O$	0.2 g.
$CaCl_2$	0.05 g.
$FeSO_4 \cdot 7\ H_2O$	0.025 g.
Distilled water	1,000 ml.
pH	4.5-4.8

The glucose was added aseptically from autoclaved solutions. The modification consisted of an increase of sugar content from 1.1 to 2.2%. It was made because Agarwal, Singh, King, and Peterson reported great decreases in cell masses of several species of yeasts, including two of those used in this study, when the sugar concentration was increased from 1.1 to 2.2%. A low cell yield has proved to be favorable to increased accumulation of lipid in the cell.

For the preparation of the inocula, tubes containing 20 ml. of Starkey's medium were inoculated with 3-mm. loopfuls from 48-hour Sabouraud's agar slant cultures. These cultures were incubated at room temperature for 48 hours and the entire contents of each tube used for the inoculation of a flask of test medium. During the 72-hour incubation period the culture flasks were agitated sufficiently to keep the cells uniformly dispersed with air that had been sterilized by passing through a 10-inch column of sterile cotton. Aeration has been shown by Starkey *(9)* to be favorable to lipid synthesis by a soil yeast. Macleod and Smedley-Maclean *(4)* have found that in the formation of fat from hexose and from acetate by yeast cells, plentiful oxygenation is an essential condition. Although neither the rate of aeration nor the oxidation-reduction potential of our cultures were measured, we felt that the relatively uniform aeration used would provide conditions favorable for lipid synthesis for all cultures. At the end of the incubation period microscopic examinations were made on samples as a check for purity.

For the determination of the dry weight of yeast produced, 10-ml. aliquots of the cultures were centrifuged; the cells were washed once with sterile distilled water, recentrifuged, dried for 10 to 12 hours in an electric oven at 60°C., and weighed.

For lipid determination the packed cells from a certain volume of the fermented liquor were resuspended in 200 ml. of 1 *N*. hydrochloric acid. The suspension was

[b]A detailed account of the origins and uses of these cultures in Germany is given in the Fiat Final Report No. 499, prepared by the Joint Intelligence Objectives Agency, Washington, D. C.

hydrolyzed for 2 hours in the steam chamber, neutralized to litmus with 1 *N*. sodium hydroxide, and filtered through a Büchner funnel using a No. 50 Whatman filter paper. The residue was dried in an electric oven at 60°C. for 2 hours and then was extracted with chemically pure absolute ethyl ether in the Soxhlet extraction apparatus.

RESULTS

Effect of nitrogen source. The lipid contents of *Torula utilis* and *Candida arborea* with various nitrogen sources are given in Tables 1 and 2, respectively. The final concentration of each substance was 0.05%. Glucose was the carbohydrate. A 0.01% yeast extract was present in addition to the nitrogen source, but when it was the sole source of nitrogen its concentration was increased to 0.05%.

TABLE 1
Effect of different sources of nitrogen on Torula utilis

Source of nitrogen	Dry yeast per 100 g. of glucose	Lipid per 100 g. of dry weight	Conversion of carbohydrate to lipid
	g.	*g.*	%
Ammonium sulfate	17.3	8.6	1.5
Asparagine	25.3	15.9	4.0
Urea	17.8	7.1	1.3
Ammonium chloride	14.2	8.9	1.3
Yeast extract	10.4	7.9	0.8
Bacto-peptone	8.8	12.2	1.2

TABLE 2
Effect of different sources of nitrogen on Candida arborea

Source of nitrogen	Dry yeast per 100 g. of glucose	Lipid per 100 g. of dry weight	Conversion of carbohydrate to lipid
	g.	*g.*	%
Ammonium sulfate	18.6	3.6	0.7
Asparagine	19.0	12.4	2.4
Urea	18.4	4.1	0.8
Ammonium chloride	9.0	5.2	0.5
Yeast extract	11.0	3.8	0.4
Bacto-peptone	10.5	10.0	1.1

In the case of *Torula utilis* the best cell mass yield was obtained with asparagine. Ammonium sulfate and urea supported growth at a level approximately 70% of that obtained with asparagine. As sole sources of nitrogen, ammonium chloride and yeast extract proved to be relatively poor supporters of growth. Bacto-peptone was the poorest supporter of growth of all the substances tested. In the case of *Candida arborea*, ammonium sulfate, asparagine, and urea gave approximately equal cell yields. Asparagine did not show the marked growth-enhancing property for *Candida arborea* that it did with *Torula utilis*. Poor cell yields were obtained with yeast extract, Bacto-peptone, and particularly with ammonium chloride.

Ammonium sulfate, urea, ammonium chloride, and yeast extract as nitrogen sources were not conducive to lipid synthesis by either *T. utilis*

or *C. arborea*. Asparagine and Bacto-peptone showed a marked stimulating effect on lipid synthesis with both yeasts. By far the highest conversion of sugar to lipid occurred with asparagine as a source of nitrogen.

With *Rhodotorula gracilis*, the effects of the six sources of nitrogen were essentially the same as with the other two yeasts. The results are given in Table 3.

TABLE 3

Effect of different sources of nitrogen on Rhodotorula gracilis

Source of nitrogen	Dry yeast per 100 g. of glucose	Lipid per 100 g. of dry yeast	Conversion of carbohydrate to lipid
	g.	*g.*	*%*
Ammonium sulfate	24.2	15.7	3.8
Asparagine	34.3	37.1	12.7
Urea	29.6	34.9	10.3
Ammonium chloride	18.6	33.8	6.3
Yeast extract	15.3	19.0	2.9
Bacto-peptone	10.6	51.1	5.4

With asparagine and urea, reasonably high yields of both cell mass and lipid were obtained. As with the other yeasts, the highest conversion of sugar to lipid occurred with asparagine; however, urea approached asparagine rather closely. Minimum cell mass and maximum lipid per cell were obtained with Bacto-peptone. A low cell yield was not necessarily associated with a high lipid content per cell, as shown by the results with yeast extract.

Rhodotorula gracilis grew better on the media used, and proved to synthesize far more fat than the other two yeasts.

Effect of carbohydrates. It was decided to use the compound which resulted in the highest conversion of glucose to fat, namely asparagine, as the source of nitrogen in experiments to determine the effect on lipid production of different carbohydrates. In the experiments with carbohydrates only *R. gracilis* was employed. The reason for this choice was that although enhancement of lipid content of *T. utilis* and *C. arborea* is possible to a certain extent by use of asparagine or Bacto-peptone in concentration of 0.05%, the increase is not sufficient to warrant a possible application of these organisms to the commercial production of lipids, at least under conditions similar to those used in the preceding experiments. These species, with their high vitamin and protein content *(1)* appear more suited for use as feed rather than as potential sources of microbial lipid.

Initially two disaccharides, sucrose and maltose, and two pentoses, xylose and arabinose, were used. The results, presented in Table 4, show that with sucrose the lipid yield was 42% higher than that obtained with glucose alone, but that the cell mass yield was 38.5% below that obtained with glucose. Maltose proved to be unsatisfactory. It appears that the yeast is not capable of utilizing this carbohydrate efficiently for either cell multiplication or lipid synthesis. Xylose, and particularly arabinose, produced slightly higher cell yields and much higher lipid yields than maltose. The results with xylose and arabinose may be of some significance

TABLE 4

Effect of different carbohydrates on lipid synthesis by Rhodotorula gracilis

Source of carbon	Dry yeast per 100 g. of sugar	Lipid per 100 g. of dry yeast	Conversion of carbohydrate to lipid
	g.	*g.*	%
Glucose	34.3	37.1	12.7
Sucrose	21.1	52.7	11.1
Maltose	2.5	8.6	0.2
Xylose	5.7	17.7	1.0
Arabinose	8.1	31.3	2.5

from the standpoint of utilizing wood hydrolyzate, which contains appreciable quantities of pentose sugars, as a cheap raw material for microbial lipid synthesis. According to Lundin *(3)*, *R. gracilis* can be adapted to a certain degree to utilize xylose. Preliminary attempts to acclimatize the yeast to Douglas fir hydrolyzate by a series of 12 serial subcultures were unsuccessful. The hydrolyzate had been subjected, in different trials, to either one of two pretreatments suggested by Peterson, Snell, and Frazier *(6)* to remove or inactivate toxic materials.

Since Bacto-peptone was found to produce very high lipid yields with glucose as the carbohydrate, it was tried with sucrose to see if any further enhancement of lipid yield could be obtained. The results are presented in Table 5. For comparative purposes the value with glucose and Bacto-peptone are given.

TABLE 5

Effects of combinations of sources of carbon and nitrogen on lipid synthesis by Rhodotorula gracilis

Source of carbon	Source of nitrogen	Dry yeast per 100 g. of sugar	Lipid per 100 g. of dry yeast	Conversion of carbohydrate to lipid
		g.	*g.*	%
Sucrose	Bacto-peptone	9.0	58.8	5.3
Sucrose	Asparagine	21.1	52.7	11.1
Glucose	Bacto-peptone	10.6	51.1	5.4

Although there was an increase of 11.6% in the lipid yield over that obtained with sucrose plus asparagine, the cell yield was of a very low order. The fact that the total conversion of carbohydrate to lipid is low, once again does not favor the use of Bacto-peptone as a source of nitrogen.

Since sucrose as a carbon source has been shown to produce cells with a higher lipid content than glucose under otherwise identical conditions, it was suspected that the increased amount of lipid per cell may have been produced by fructose or a fructose derivative resulting from the breakdown of sucrose. In order to test this point, experiments were devised a, to find if the use of equal parts of glucose and fructose would result in a lipid content similar to that obtained with sucrose, and b, to confirm the augmenting role of fructose with data obtained by the use of increasing amounts of this carbohydrate and decreasing amounts of glucose, so that

the total carbohydrate concentration was always 2.2%. The source of nitrogen in these experiments was asparagine.

The results of the two experiments are given in Tables 6 and 7. The cell mass and lipid yields resulting from equal parts of glucose and fructose were very similar to those obtained with sucrose alone. Data in Table 7 show that, as the proportion of fructose increased, the cell mass progressively decreased, and the lipid yield increased. Maximum lipid yield and minimum cell mass were obtained with fructose alone.

TABLE 6

Effect of substitution of glucose and fructose for sucrose on lipid synthesis by Rhodotorula gracilis

Source of carbon	Dry yeast per 100 g. of sugar	Lipid per 100 g. of dry yeast	Conversion of carbohydrate to lipid
	g.	*g.*	%
Glucose, 1.1%, plus fructose, 1.1%	20.7	49.8	10.6
Sucrose, 2.2%	21.1	52.7	11.1
Glucose, 2.2%	34.3	37.1	12.6

TABLE 7

Effect of proportion of glucose and fructose on lipid synthesis by Rhodotorula gracilis

Source of carbon	Dry yeast per 100 g. of sugar	Lipid per 100 g. of dry yeast	Conversion of carbohydrate to lipid
	g.	*g.*	%
Glucose	34.3	37.1	12.6
Glucose, 1.7%, plus fructose, 0.5%	30.2	42.8	12.9
Glucose, 1.2%, plus fructose, 1.0%	27.2	48.8	13.4
Glucose, 0.5%, plus fructose, 1.7%	25.6	55.0	14.1
Glucose, 0.2%, plus fructose, 2.0%	23.8	57.9	13.8
Fructose alone, 2.2%	22.6	60.3	13.6

SUMMARY

Evidence is presented that the sources of nitrogen and carbohydrate are of primary importance in the cultivation of yeasts for lipid synthesis.

Six nitrogen sources, namely, ammonium sulfate, asparagine, urea, ammonium chloride, higher concentrations of yeast extract, and Bacto-peptone were tested for ability to support cell multiplication and lipid synthesis by *Torula utilis* and *Candida arborea*. Asparagine was found to be by far the best from the standpoint of both cell mass and lipid yields. The lipid content of the two yeasts grown on glucose-asparagine medium was of the order of 15.9% and 12.4%, respectively. Ammonium sulfate, urea, ammonium chloride, and higher concentrations of yeast extract gave poorer yields of cell mass and of lipid than did asparagine. Bacto-peptone gave good yields of lipid per cell, but very poor yields of cell substance.

The lipid yields of *Rhodotorula gracilis* with asparagine, urea, and ammonium chloride were 37.1%, 34.9%, and 33.2%, respectively. Ammonium sulfate and higher concentrations of yeast extract were unsatisfactory with respect to both cell mass and lipid yields. As in the case of other

yeasts, Bacto-peptone gave a high lipid yield per cell but an extremely poor yield of cell mass.

As sources of carbohydrate for lipid synthesis by *R. gracilis*, fructose and sucrose were found to be superior to glucose from the standpoint of lipid content per cell. Xylose, arabinose, and maltose gave poor yields of cell material and lipids. With varying the concentrations of glucose and fructose in a manner to give a total concentration of 2.2%, cell yield decreased and cellular lipid content increased as the fructose concentration increased.

A high cellular lipid content was often, but not always, associated with a low cell yield.

LITERATURE CITED

1. Agarwal, P. N., Singh, K., King, P. S., and Peterson, W. H. Yields and vitamin contents of food yeasts grown on different kinds of molasses. *Arch. Biochem.*, **14**, 105 (1947).
2. Kleinzeller, A. Fat formation in *Torulopsis lipofera*. *Biochem. J.*, **38**, 480 (1944).
3. Lundin, H. Fat synthesis by micro-organisms and its possible applications in industry. *J. Inst. Brewing*, **56**, 17 (1950).
4. Macleod, L. D., and Smedley-Maclean, I. The carbohydrate and fat metabolism of yeast. *Biochem. J.*, **32**, 1571 (1938).
5. Nilsson, R., Eneбo, L., Lundin, H., and Myrbäck, K. Microbial synthesis of fat with *Rhodotorula glutinis*. *Svensk. Kem. Tid.*, **55**, 41 (1943), *C. A.*, **38**, 58769 (1944).
6. Peterson, W. H., Snell, J. F., and Frazier, W. C. Fodder yeast from wood sugar. *Ind. Eng. Chem.*, **37**, 30 (1945).
7. Rippel, A. Biologische Fettgewinnung durch Hefen in Luftungsverfahren. *Naturwissenschaften*, **31**, 248 (1943).
8. Skinner, C. E., Emmons, C. W., and Tsuchiya, H. H. *Molds, Yeasts and Actinomycetes*. 1947, John Wiley and Sons, New York, N. Y.
9. Starkey, R. L. Lipid production by a soil yeast. *J. Bact.*, **51**, 33 (1946).

THE B VITAMINS IN RAW AND COOKED LAMB. I. THIAMINE

ETHELWYN B. WILCOX AND LEORA S. GALLOWAY
Department of Foods and Nutrition, Utah Agricultural Experimental Station, Logan, Utah

(Manuscript received August 6, 1951)

Cross breed lambs are being studied by Bell, Madsen, Bennett, Madsen, and Schmutz *(1)* at Utah State Agricultural College to improve the meat producing abilities of range sheep. High quality rams are being bred to range ewes. The thiamine, riboflavin, and niacin content of lambs selected from the cross breeds being developed was determined in this laboratory. These lambs have a known nutritive history. Studies on thiamine content of lamb whose nutritive history was unknown have been made by McIntire, Schweigert, and Elvehjem *(5)* and Cover and Dilsaver *(3)*. The lambs used by Noble, Spear, and Turnbull *(7)* and Noble, Gordon, and Nelson *(6)* had a known breed and feed history.

A study of the thiamine content of lamb chops, legs, shoulders, and stew meat is reported herein.

EXPERIMENTAL PROCEDURE

The lambs were selected at random from the large experimental herd of sheep in southern Utah, where they had been raised under good range conditions. First and second cross lambs from Rambouillet ram x range ewe (R_1 and R_2) and from Columbia ram x range ewe (C_1 and C_2) and first cross lambs from Targhee ram x C_1 ewes (T_1) were studied. The lot of lambs that was taken directly from the range is designated as grass-fat, while the other lot was fed for 92, 93, and 97 days for the 1947, 1948, and 1949 lambs, respectively, on a grain mixture of dry molasses, beet pulp, whole barley or wheat, salt, and steamed bonemeal plus alfalfa hay. The grass-fat lambs were approximately five and one-half months old, while the fed lambs were approximately nine months old.

The uniformity of the lambs was shown by the similarity of their carcass grades and weights (Table 1). After the carcasses were aged for 7 days under commercial conditions, they were cut into shoulder roasts, leg roasts, and 1-inch thick loin and rib chops, with the rest of the lean meat being used for stew meat. All cuts of meat were quick-frozen and cooking tests were completed with samples taken for analyses within a month. Corresponding cuts from the left and right sides were used for the raw and cooked samples, respectively. Duplicate determinations for thiamine, moisture, crude fat, and total nitrogen were made on samples from the muscle (longissimus dorsi) in the raw and cooked chops and on samples from the raw and cooked legs, raw shoulders, and a 500-g. lot of stew meat after grinding. The fat on the leg or shoulder was ground with the lean. The stew meat had most of the fat removed before it was cut into 1-inch cubes and divided into 500-g. portions for freezing.

The muscle of the chop was cut out, weighed, and then replaced in the chop for panbroiling at a temperature of 230° to 266° F.(110° to 130°C.) until medium done. After cooking the chop, the muscle was weighed and analyzed. The legs were roasted at an oven temperature of 325°F.(163°C.) to an internal temperature of 180°F.(82°C. medium done).

In cooking the stews, 500-g. portions of stew from each animal were cooked with 40 ml. and with 250 ml. of tap water for 15 minutes in a pressure saucepan at 250°F. (121°C.). Then the stew meat was lifted out with a spoon and weighed before grinding for analyses. The broth and coagulated proteins were placed in the refrigerator to harden the fat, which was removed before analyzing the broth.

TABLE 1

Grade and weight of lamb

Lamb	Number of lambs	Carcass grade	Carcass weight
Grass-fat lambs			
			lbs.
1947. Columbia 1st cross (C_1)	4	Good	46-51
Rambouillet 1st cross (R_1)	3	Good	47-52
Rambouillet 1st cross (R_1)	1	Commercial	44
1948. Columbia 1st cross (C_1)	4	Good	48-57
Rambouillet 1st cross (R_1)	4	Good	44-48
1949. Columbia 2nd cross (C_2)	2	Good	44-45
Columbia 2nd cross (C_2)	5	Choice	44-54
Columbia 2nd cross (C_2)	1	Prime	52
Rambouillet 2nd cross (R_2)	4	Good	45-48
Rambouillet 2nd cross (R_2)	4	Choice	49-53
Targhee 1st cross (T_1)	2	Good	41-44
Targhee 1st cross (T_1)	5	Choice	47-52
Targhee 1st cross (T_1)	1	Prime	53
Fed lambs			
1947. Columbia 1st cross (C_1)	3	Good	46-52
Columbia 1st cross (C_1)	1	Commercial	47
Rambouillet 1st cross (R_1)	2	Good	47-54
Rambouillet 1st cross (R_1)	2	Commercial	43-48
1948. Columbia 1st cross (C_1)	3	Good	52-57
Columbia 1st cross (C_1)	7	Commercial	43-52
Rambouillet 1st cross (R_1)	2	Good	49-52
Rambouillet 1st cross (R_1)	8	Commercial	43-51
1949. Columbia 2nd cross (C_2)	3	Good	46-49
Columbia 2nd cross (C_2)	5	Commercial	44-51
Rambouillet 2nd cross (R_2)	3	Good	50-53
Rambouillet 2nd cross (R_2)	5	Commercial	44-49
Targhee 1st cross (T_1)	6	Good	44-53
Targhee 1st cross (T_1)	2	Commercial	42-49

Thiamine was determined by a modification of the method of Conner and Straub *(2)*. The meat was ground with an electric grinder, and 100-g. samples were blended in a Waring Blendor. After heating for 60 minutes in a boiling water bath followed by cooling, a mixture of Clarase and papain was added. The sample was incubated with the enzymes for 20 to 24 hours at 45°C.(113°F.). Aliquots of the sample were used directly for oxidation of the thiamine to thiochrome for reading.

Nitrogen determinations were made by the Kjeldahl method.

RESULTS AND DISCUSSION

The average thiamine values for the raw cuts of lamb on the moist basis ranged from 0.096 mg. per 100 g. for shoulder or stew meat to 0.192 for the chops (Table 2). These values are within the range of those reported for lamb by McIntire, Schweigert, and Elvehjem *(5)* and Noble, Spear, and Turnbull *(7)*, 0.18 to 0.20 mg. per 100 g. for leg; Noble, Gordon, and Nelson *(6)*, 0.10 mg. for shoulder; and McIntire, Schweigert, and Elvehjem *(5)*, and Cover and Dilsaver *(3)*, 0.14 to 0.17 for stew meat. In a preliminary study on Rambouillet lamb by Wilcox, Morrell, and Neilson *(8)* the chops averaged 0.141 and 0.185 mg. per 100 g. and stew meat 0.098

TABLE 2

Thiamine content of raw lamb

No. of lambs	Thiamine content						
	Chops		Shoulders		Legs		Stew[a]
	Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis	Moist basis
	mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
Grass-fat lambs							
1947 C_1 4	0.168	0.784	0.095	0.580	0.162	0.820	0.118
1948 C_1 4	0.217	0.978	0.142	0.762	0.198	0.992	.....
Average	0.192	0.881	0.118	0.671	0.180	0.906	0.118
1947 R_1 4	0.168	0.771	0.104	0.558	0.154	0.854	0.117
1948 R_1 4	0.204	0.926	0.116	0.671	0.180	0.891	.....
Average	0.186	0.848	0.110	0.614	0.167	0.872	0.117
1949 C_2 10	0.168	0.710	.....	.....	0.133[b]	0.685	.....
1949 R_2 10	0.155	0.669	.....	.....	0.123[b]	0.613	.....
1949 T_1 8	0.165	0.728	.....	.....	0.142	0.739	.....
Fed lambs							
1947 C_1 4	0.149	0.700	0.126	0.634	0.160	0.749	0.096
1948 C_1 10	0.134	0.611	0.091	0.554	0.122	0.649	.....
Average	0.142	0.656	0.108	0.594	0.141	0.699	0.096
1947 R_1 4	0.136	0.650	0.115	0.578	0.148	0.706	0.098
1948 R_1 10	0.136	0.618	0.077	0.455	0.108	0.556	.....
Average	0.136	0.634	0.096	0.516	0.128	0.631	0.098
1949 C_2 8	0.141	0.634	.....	.....	0.115	0.601	.....
1949 R_2 8	0.162	0.711	.....	.....	0.120	0.585	.....
1949 T_1 8	0.141	0.618	.....	.....	0.103	0.531	.....

[a] Two animals used for analyses of stews.
[b] Eight animals used for analyses of legs.

and 0.088 for grass-fat and lot fed animals, respectively. These values for stew are slightly lower than those for the R_1 lambs in this study.

Although the chops contained more thiamine than the legs on the moist basis, the values for the dry, fat-free leg muscle slightly exceeded those of the chops in most cases. The difference in fat content would account for the chops being higher on the moist basis. Fat content, however, did not account for the lower values of the shoulders. The differences between thiamine content on the moist or dry, fat-free basis of the shoulders and of the legs or chops were highly significant.

Four C_1 and R_1 lambs were used the first year with additional C_1 and R_1 lambs being studied the next year. The numbers in each group were increased starting with the fed group of 1948 to increase the validity of the data. The quite wide variability that may occur between animals of the same age, breed, and feeding was noted previously by Wilcox, Morrell,

and Neilson *(8)*. Cover, Dilsaver, and Hays *(4)* found significant variations in the B vitamin content of lamb from carcass to carcass.

When the values for the two cross breeds, C_1 and R_1, were compared, analyses of the data showed that the tissues of the C_1 lambs contained significantly more thiamine than did the R_1 lamb tissues. However, this was not true for the second cross or C_2, R_2, and T_1 lambs, which showed no significant differences.

The different cuts of the grass-fat lambs contained more thiamine than did the same cuts from the fed lambs for the same year with one exception (R_2 chops) on either the moist or dry, fat-free basis. Values were also calculated on the basis of the protein content of the tissue. When the thiamine content of the R_2 chops was thus calculated, the value for the grass-fat lambs was higher than for the fed lambs (0.810 and 0.774 mg. per 100 g., respectively). Hence, the R_2 lambs showed the same trend as the other animals when values were calculated on the basis of their protein content.

A more direct comparison of the effect of feeding on the thiamine content of lamb was made by combining all values for the C_1 and R_1 lambs (Table 3). The grass-fat lamb tissues contained more thiamine than did

TABLE 3

Combined thiamine values of C_1 and R_1 lambs

Raw muscle	Grass-fat lambs	Fed lambs	Weighted average
	Thiamine, mg. per 100 g.	*Thiamine, mg. per 100 g.*	
Chops	0.189	0.137	0.156
Legs	0.174	0.126	0.143
Shoulders	0.114	0.094	0.102
Weighted average	0.159	0.119	.....

those of the fed lambs, the difference being highly significant. The difference between the values of the shoulders of the grass-fat and fed lambs was much smaller than for the chops and legs. This interaction was significant.

Similar analyses of the data for the C_2, R_2, and T_1 lambs also showed highly significant differences between the thiamine content of the tissues of the grass-fat and fed lambs.

The average retention of thiamine in panbroiled chops was similar for either the C_1 or R_1 chops of the grass-fat lambs (68.0 and 68.4%) or of the fed lambs (63.0 and 61.3%, respectively) when the retention was calculated back to the original weight of the muscle (Table 4). Differences were slightly greater on the dry, fat-free basis, however they were not significant. Slightly less thiamine was retained in the roasted legs (63.3 to 49.2%). Variations between values for either the grass-fat or fed C_1 and R_1 lambs were small. Retention in the cooked chops or legs of the grass-fat lambs was slightly higher than in the corresponding cut of the fed lambs. Similar retention values for thiamine in cooked chops were obtained by Wilcox, Morrell, and Neilson *(8)*. McIntire, Schweigert, and Elvehjem *(5)* reported thiamine retentions of 53 and 56% in roast leg of lamb, while Noble, Spear, and Turnbull *(7)* found that 66% was retained.

TABLE 4

Thiamine content of cooked chops and legs of lamb

No. of lambs	Moist basis		Dry, fat-free basis	
	Cooked meat		Cooked meat	
	Thiamine	Retention[c]	Thiamine	Retention[c]
Chops				
	mg. per 100 g.	%	*mg. per 100 g.*	%
Grass-fat lambs				
1947 C_1 4	0.164	77.3	0.603	77.0
1948 C_1 4	0.190	58.8	0.617	63.0
Average	0.177	68.0	0.610	70.0
1947 R_1 4	0.171	74.1	0.617	79.4
1948 R_1 4	0.191	62.7	0.615	66.9
Average	0.181	68.4	0.616	73.2
Fed lambs				
1947 C_1 4	0.144	66.3	0.478	67.1
1948 C_1 10	0.130	59.7	0.387	64.7
Average	0.137	63.0	0.432	65.9
1947 R_1 4	0.133	68.3	0.430	68.5
1948 R_1 10	0.118	54.3	0.354	57.2
Average	0.126	61.3	0.392	62.8
Legs				
Grass-fat lambs				
1948 C_1 4	0.171	63.3	0.672	67.8
1948 R_1 4	0.142	56.4	0.540	60.1
Fed lambs				
1948 C_1 10	0.099	49.2	0.353	54.0
1948 R_1 10	0.095	55.2	0.322	58.5

[c] Calculated back to original weight of muscle analyzed.

The size of the chop would affect the total amount of thiamine present in an ordinary serving. These chops which were cut one inch thick had an average weight of 145 g. for the grass-fat and 155 g. for the fed lambs. Chops from either lot of lambs contained the same weight of lean, hence, the fat and bone weighed more in the fed lambs. One-third of the weight was lean and two-thirds fat and bone in the chops from the grass-fat lambs. The thiamine content of a serving of one chop averaged 0.06 and 0.04 mg. for the grass-fat and fed lambs, respectively. A serving of roast leg averaging 70 g. contained 0.109 and 0.068 mg. of thiamine for the grass-fat and fed lambs, respectively.

More thiamine was found in the stew meat of the stews cooked in a small amount of water (Table 5), while the reverse was true for that retained in the broth. This unequal distribution of thiamine between the meat and the broth resulted in similar retentions in total thiamine for all stew meat and broth. Differences between the combined stew meat and broth when the small or large amounts of water were used were too small to be significant. Cover and Dilsaver *(3)* also did not find significant

differences for the total thiamine retained for large and small amounts of water.

Approximately 50% of the thiamine was retained in the combined broth and stew for all stews of either lots of the C_1 and R_1 lambs. Similar values were reported by Wilcox, Morrell, and Neilson *(8)* (50 and 58%); by McIntire, Schweigert, and Elvehjem *(5)* (50%); and by Cover and Dilsaver *(3)* (60% on the dry, fat-free basis for stews cooked under similar conditions).

TABLE 5

Thiamine content and retention in cooked lamb stew

	Grass-fat lambs		Fed lambs	
	1947 C_1	1947 R_1	1947 C_1	1947 R_1
In total stew meat, mg.				
Raw	59.38	58.63	47.88	48.88
Cooked				
Small	16.32	17.25	14.40	15.54
Large	13.12	13.65	10.41	12.66
In total broth, mg.				
Small	11.70	12.45	9.32	8.88
Large	15.78	16.60	13.54	13.79
In total stew and broth, mg.				
Small	28.02	29.70	23.72	24.43
Large	28.90	30.25	23.94	26.45
Retention in stew meat, %				
Small	27.5	29.8	30.3	31.6
Large	22.4	23.2	21.9	26.0
Retention in broth, %				
Small	19.4	21.4	19.2	18.6
Large	26.7	28.3	28.0	28.4
Retention in stew and broth, %				
Small	46.9	51.2	49.5	50.2
Large	49.1	51.5	49.9	54.4

SUMMARY

The thiamine contents of raw and cooked chop, leg, and stew and of raw shoulder of first cross Rambouillet and Columbia grass-fat and fed lambs were studied. Raw chops and legs of second cross Rambouillet and Columbia and first cross Targhee lambs were also studied.

The average thiamine values for the raw lamb ranged from 0.096 mg. per 100 g. for shoulder or stew to 0.192 for chops, the difference being highly significant. The chops and legs had similar thiamine contents.

The C_1 lamb tissues contained significantly more thiamine than did those of the R_1 lambs. However, differences between the C_2, R_2, and T_1 lambs were not significant. The grass-fat lamb tissues contained significantly more thiamine than did those from the fed lambs.

Retention of thiamine for the panbroiled chops and roasted legs were similar for the C_1 or R_1 lambs. Values for the chops varied from 63 to

68% and for the legs from 49 to 63%. The percent of thiamine retained after cooking stews in a small or large amount of water was similar with an average of 50%.

LITERATURE CITED

1. Bell, T. D., Madsen, M. A., Bennett, J. A., Madsen, L. L., and Schmutz, D. C. Rambouillet and Columbia-Rambouillet lambs on the range and in the feed lot. Utah Agr. Expt. Sta., *Bull.* **341** (1950).
2. Conner, R. T., and Straub, G. J. Combined determinations of riboflavin and thiamine in food products. *Ind. Eng. Chem., Anal. Ed.*, **13**, 385 (1941).
3. Cover, S., and Dilsaver, E. M. Retention of the B vitamins in beef and lamb after stewing. II. Thiamine. *J. Am. Dietet. Assoc.*, **23**, 613 (1947).
4. Cover, S., Dilsaver, E. M., and Hays, R. M. Variation among lamb carcasses in the B vitamin content of meat. *Science*, **105**, 364 (1947).
5. McIntire, J. M., Schweigert, B. S., and Elvehjem, C. A. The retention of vitamins in veal and lamb during cooking. *J. Nutrition*, **26**, 621 (1943).
6. Noble, I., Gordon, J., and Nelson, D. The effect of roasting to different interior temperatures upon the thiamine and riboflavin content of rolled lamb shoulders. Minn. Agr. Expt. Sta., *Progress Note*, Misc. Series, **555** (1946).
7. Noble, I., Spear, J., and Turnbull, F. The effect of roasting to different interior temperatures upon the thiamine content of leg of lamb. Minn. Agr. Expt. Sta., *Progress Note* (1944).
8. Wilcox, E. B., Morrell, K. E., and Neilson, A. M. The effect of various factors on the thiamine and riboflavin content of lamb chops and stews. Utah Agr. Expt. Sta., *Progress Note*, **6** (1947).

THE EFFECT OF PARTICLE SIZE ON THE NUTRITIONAL CHARACTERISTICS OF UNIFINE FLOUR[a]

MARY CORBETT STEVENS,[b] JANICE F. SIEBURTH,[b] TOM WAHL,[c] AND BARBARA McLAREN[b]
The State College of Washington, Pullman, Washington

(Manuscript received August 17, 1951)

The Division of Industrial Research of the State College of Washington has developed an experimental high-speed rotary flour mill which grinds the entire wheat berry *(16)*. Grinding is accomplished in one pass through the mill, and no screening or other treatment of the flour has been found necessary.

It has been named Unifine flour by virtue of the fact that it is ground to a fineness and uniformity of high degree in comparison with whole-wheat flour milled by the roller process. The flour is a light-fawn color, and bread produced from it is of good volume, texture, uniformity, and flavor.

Unifine flour not only contains the endosperm, but also the wheat germ, aleurone layer, and bran. The proteins of these latter components are composed of a different mixture of amino acids from that of the endosperm and have a higher biological value *(5)*. Wheat is markedly deficient in two amino acids, valine and lysine. Of the two wheat products, white flour is more deficient in these amino acids than whole wheat flour. In recent work, the biological value of the wheat germ and bran have been studied separately. Hove and Harrel *(10)* showed that wheat germ had a higher value than casein (as measured by protein efficiency) and 2½ times the value of the so-called average American diet. In an investigation of the biological effect of adding bran to a white flour diet, Chick, Cutting, Martin, and Slack *(7)* obtained an increase in nutritive value with increasing amounts of bran.

The relative digestibility of whole wheat and white breads was studied as early as 1883 by Rubner *(17)*. He used 30, 70, and 100% of the wheat kernel and found the protein digestibility to be 79.3, 75.4, and 69.5%, respectively. Macrae, Hutchison, Irwin, Bacon, and McDougall *(14)*, using humans, studied the availability of the constituents of white and whole wheat flours, and the effect on protein digestibility of the fineness to which whole wheat flour was ground. The protein of white bread was more completely digested than that of the whole wheat bread. Using a coarse and fine whole wheat flour, no appreciable differences in digestibility were found.

Since wheat carries a good supply of the B vitamins, it was of interest to determine whether more complete grinding, resulting in smaller par-

[a] Scientific paper No. 1052, Project 850, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman, Washington.

[b] Department of Home Economics.

[c] Division of Industrial Research.

ticle size, would alter the availability of the vitamins. Thiamine was chosen as an indicator of the B vitamins.

Previous work has indicated that fineness of grind as produced in the Unifine mill improved the baking characteristics of whole wheat flour *(8, 16)*. Consumer acceptance studies by McLaren and Harrington *(13)*, demonstrated that the palatability had also been considerably increased. For this reason, it was of interest to learn the nutritive value of the proteins of Unifine flour, whether the Unifine product was more or less digestible, and whether the thiamine was more available than in commercial whole wheat flour.

MATERIALS AND METHODS

Unifine flour of three degrees of fineness (G-1, very fine, motor speed 1200 r.p.m.; G-3, medium, motor speed 900 r.p.m.; G-5, coarse, motor speed 600 r.p.m.) milled from wheat of 14.8% protein was used as well as commercial whole wheat and patent flours.

Assay method for growth and protein efficiency. The method of Osborne and Mendel *(15)* for the measurement of protein efficiency (grams gain in body weight per gram protein consumed) was used to compare the Unifine, (G-3) whole wheat flour, patent flour, egg powder, and casein diets. Two series of experiments 1 and 2 were used to measure the protein efficiency ratio. Casein, as a reference, and Unifine were included in both. Egg powder was used as a reference as well as casein in the first experiment. Patent and commercial whole wheat flours were compared in experiment 2 with Unifine flour and casein.

TABLE 1

Composition of diets containing 10% protein

	Unifine	Whole wheat flour	Patent flour	Egg powder	Casein
Unifine flour	72.2	.....	.....	.....	.....
Whole wheat flour	.....	67.1	.....	.....	.....
Patent flour	.....	.....	83.3	.....	.....
Egg powder	.....	.....	.....	22.6	.....
Casein	.....	.....	.....	.....	12.3
Hubbell's salts[4]	4.0	4.0	4.0	4.0	4.0
Fat (Wesson Oil)	8.8	8.6	8.6	1.4	10.0
Starch	15.0	20.3	4.1	72.0	73.7
Total	100.0	100.0	100.0	100.0	100.0

[4] Hubbell, Mendel, and Wakeman *(11)*.

Rats were fed ad libitum diets adjusted to contain 10% protein and 10% fat (Table 1). Nitrogen was estimated by the Kjeldahl method *(1)* and the value obtained multiplied by a factor of 5.7 for all flours and by 6.25 for egg powder and casein in calculating protein content. Each rat received as a supplement 3 times weekly the following vitamin solution: thiamine hydrochloride, 50 γ; riboflavin, 50 γ; pyridoxine hydrochloride, 50 γ; calcium pantothenate, 250 γ; nicotinic acid, 580 γ; inositol, 130 γ; choline hydrochloride, 20 γ; p-aminobenzoic acid, 250 γ; vitamin A, 330 I.U.; vitamin D, 33 I.U.; and mixed tocopherols, 100 γ.

Young rats, housed individually, were maintained on the diets for a 6-week period. Each rat was weighed once a week.

Assay method for digestibility. Digestibility of 3 grinds of Unifine, the coarsest (G-5), the intermediate (G-3), and the finest (G-1), was compared with whole wheat and patent flours using diets compounded as previously described (Table 1). Chick's method *(5, 6)* was used to determine the digestibility. Adult rats were housed individually in metabolism cages so designed that the feces and urine could be kept separate.

Diets were run consecutively on a group of 6 animals for periods of 11 days. This included a 4-day adjustment period and a 7-day collection period. Nitrogen content of

diets and feces was determined by the Kjeldahl method *(1)*. The coefficient of apparent digestibility was determined by the following formula:

$$\text{Coefficient of apparent digestibility} = \frac{\text{N intake} - \text{fecal N}}{\text{N intake}} \times 100.$$

To determine apparent digestibility of the fiber, both the flour and feces were analyzed by the A.O.A.C. method *(1)* for crude fiber.

Assay method for thiamine availability. Weanling white albino rats which varied in weight from 76-89 g. were used. At the beginning of the assay period, the rats were put into individual wire cages with raised floors, which were cleaned 3 times a week. The rats were fed a thiamine-free diet and depleted, following the A.O.A.C. *(1)* method for thiamine assay. Food and water were supplied ad libitum. The thiamine-free diet is as follows:

	Percent
Casein	22.0
Sucrose	61.5
Hubbell salts *(11)*	2.5
Crisco	9.0
Autoclaved yeast	5.0

The same vitamin solution without thiamine was given to these rats as mentioned earlier.

Unifine flour of fine (G-1), medium (G-3), and coarse (G-5) grinds, milled from the same wheat, was compared with a commercial whole wheat flour. Thus, the 3 samples of Unifine contained potentially the same amount of thiamine.

The rats were divided into groups of approximately 9 and weighed 3 times a week for 6 weeks. Four groups were used for the standard curve determination. These were given orally: 0 γ, 15 γ, 30 γ, and 45 γ, respectively, of pure thiamine hydrochloride 3 times a week. The assay groups were given supplements of the various grinds of flours at 2 levels: 4 groups were fed 1 g. of the different flour samples 3 times weekly, and 4 other groups were given 3 g. at the same intervals.

RESULTS AND DISCUSSION

Growth and protein efficiency. The data on the ability of the various diets to support rat growth are summarized in Table 2. Unifine (G-3) occupied an intermediate position, being neither so poor as white and whole wheat flours, nor so good as egg powder and casein.

TABLE 2

Comparative rat growth and protein efficiency of various diets with Unifine diet fed at 10% protein level

Diet	Experiment	No. of animals	Weekly gain	Total gain*	Average food intake	Protein efficiency ratios*
	no.		g.	g.	g.	
Unifine flour	1	10	8.7	52± .78	457	1.14±.01
Unifine flour	2	10	10.0	60± .73	440	1.36±.01
Whole wheat flour	2	10	5.7	34± .85	353	0.96±.02
Patent flour	2	10	5.2	31± .62	332	0.94±.02
Egg powder	1	10	21.2	127±2.43	495	2.57±.03
Casein	1	10	13.7	82± .87	471	1.74±.02
Casein	2	10	9.7	58±1.01	347	1.67±.02

* Includes Standard Deviation.

These results compare closely with those obtained by other workers. Whole wheat flour, when fed to rats at a 10% protein level, gave a protein efficiency value of 1.03 *(3)*. These workers reported a value of 2.6 for an egg powder 10% protein diet in an earlier investigation *(2)*. Block and

TABLE 3

Comparative digestibility of three grinds of Unifine flour with patent and whole wheat flours using nitrogen and crube fiber determinations

Diet	Initial weight	Food eaten	Nitrogen eaten	Dry weight of feces	Nitrogen in feces	Coefficient of apparent digestibility	Crude fiber in			Percent digestion of fiber by rats
							Flour	Diet	Feces	
	g.	*g.*	*g.*	*g.*	*g.*		%	%	%	
Patent flour	321	115	2.162	7.04	0.334	84.5	0.3	....	....	....
Whole wheat flour	335	102	1.928	9.64	0.374	80.6	2.7	1.5	13.1	18.8
Unifine G-1 flour	325	103	1.844	12.96	0.447	75.8	2.1	1.5	10.4	12.6
Unifine G-3 flour	295	138	2.444	16.75	0.638	73.9	2.2	1.5	10.6	20.2
Unifine G-5 flour	346	96	1.757	9.73	0.375	78.7	2.2	1.8	12.7	31.8

Mitchell *(4)* report that the average protein efficiency obtained by various workers in 5 experiments was 2.0 when casein diets were fed.

Digestibility. All three grinds of Unifine flour (Table 3) were lower in digestibility than commercial whole wheat flour. These values agree with mean values for patent and whole wheat flours compiled by Wright *(19)* from the reports of several investigators who found an average apparent digestibility of 86% for white bread and 81% for a whole wheat product. Macrae, Hutchinson, Irwin, Bacon, and McDougall *(14)* who fed diets of white, fine whole wheat, and coarse whole wheat breads to humans, report values of 91.1, 85.3, and 85.1%, respectively.

The results of the crude fiber determinations on the flour, the diets, and the feces of the rats are shown in Table 3. There is a progressive increase in the amount of fiber present as one proceeds from patent, to Unifine, to commercial whole wheat flours. The amount of fiber left in the feces of rats on diets made up from these flours, follows the same pattern. It is interesting to note that the crude fiber seems more digestible in the coarser grinds.

TABLE 4

Growth of rats on various levels of thiamine

Diet	Weight (grams) per week						
	0	1	2	3	4	5	6
Standard curve:							
0 γ thiamine	84	81	71	64	67	70	53[f]
15 γ thiamine	85	87	99	127	146	154	167
30 γ thiamine	83	89	116	145	161	175	186
45 γ thiamine	86	94	123	146	162	177	185
First level: 1 g.							
Unifine G-1 flour	82	89	111	131	141	150	154
Unifine G-3 flour	81	88	102	124	139	148	154
Unifine G-5 flour	76	89	101	122	136	149	155
Whole wheat flour	86	98	114	128	141	152	158
Second level: 3 g.							
Unifine G-1 flour	89	95[g]	115	143	160	174	181
Unifine G-3 flour	82	93	116	143	158	174	185
Unifine G-5 flour	86	100	125	146	163	175	182
Whole wheat flour	85	95	120	140	157	169	175

[f] Mortality amounted to 86% in this group.
[g] Mortality amounted to 11% in this group.

Thiamine availability. The rat-curative thiamine assay method was chosen to measure the availability of the B vitamins as affected by fineness of grind, because thiamine is susceptible to destruction by heat, and the thiamine deficiency in rats is very clear cut and quick to produce. In order to obtain the particle size such as is present in Unifine, some heat is produced in the flour *(16)* which could destroy some of the thiamine.

Johnson *(12)* suggested that the rat-curative method for thiamine assay is more specific than rat growth, and has an accuracy of the order of 10%. Whitney, Herren, and Westerman *(18)* found that rat-curative method used for ground wheat samples checked with the average value obtained by other workers using the thiochrome procedure.

All rats except one on the zero level of thiamine died by the end of the fifth week. This group showed signs of acute thiamine deficiency (polyneuritis). There was a progressive weight decrease (Table 4). On the diet supplemented with 15 γ of thiamine, the growth rate of the rats was slow, while the administration of the two higher levels resulted in optimum growth curves which were about the same. Thus, the requirement for thiamine for the rat for optimum growth on this diet, was around 70-90 γ per week, which compares favorably with the values reported by Hawk, Oser, and Summerson *(9)*.

When each of the 4 different samples were fed at 2 levels, 1 and 3 g., all groups of rats grew at approximately the same rate, indicating that there was no appreciable difference in availability of thiamine among the flours of varying degrees of fineness. The rate of growth of the rats on the 1-g. level of flour was slightly less than the standard thiamine group supplemented with 15γ of thiamine, while those on the 3-g. level of flour grew at a rate approximating that of the animals on the 2 higher levels of thiamine, 30 and 45 γ.

SUMMARY

The finer grind of Unifine has changed the baking characteristics and some of the physical properties of whole wheat flour. Growth studies with rats have shown that Unifine resulted in a better growth at a 10% level of protein than a commercial whole wheat flour. These data brought up the question of whether the digestibility of the flour and the availability of the nutrients were increased due to the decreased particle size. However, using nitrogen and crude fiber methods to determine the extent of digestion in the rats, the coarser whole wheat flour seemed to be more digestible than the finer; and thiamine, chosen as indicator of the B vitamins, appeared to be equally available in flours of several degrees of fineness.

LITERATURE CITED

1. Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th Ed., 1945, Assoc. Official Agr. Chemists, Washington, D. C.
2. Barnes, R. H., Maack, J. E., Knights, M. J., and Burr, G. O. Measurement of the growth promoting quality of dietary protein. *Cereal Chem.*, **22**, 273 (1945).
3. Barnes, R. H., and Bosshardt, D. K. The evaluation of protein quality in the normal animal. *Ann. N. Y. Acad. Sci.*, **47**, 273 (1946).
4. Block, R. J., and Mitchell, H. H. The correlation of the amino acid composition of the proteins with their nutritive value. *Nutrition Abstracts and Revs.*, **16**, 249 (1947).
5. Chick, Harriette. Biological value of the proteins contained in wheat flours. *Lancet*, **242**, 405 (1942).
6. Chick, Harriette, Copping, A. M., and Slack, E. B. Nutritive values of wheat flours of different extraction-rate. *Lancet*, **250**, 196 (1946).
7. Chick, Harriette, Cutting, M. E. M., Martin, C. J., and Slack, E. B. Observations on the digestibility and nutritive value of the nitrogeneous constituents of wheat bran. *Brit. J. Nutrition*, **1**, 161 (1948).
8. Corbett, Mary Nasmith. A study of unifine flour. M. S. Thesis. Washington State College (1949).
9. Hawk, Philip B., Oser, Bernard L., and Summerson, William H. *Practical Physiological Chemistry*. 12th Ed., 1947, The Blakiston Company, Philadelphia.
10. Hove, E. L., and Harrel, C. G. The nutritive value of wheat germ protein. Cereal Chem., **20**, 141 (1943).
11. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J. A new salt mixture for use in experimental diets. *J. Nutrition*, **14**, 273 (1937).

12. JOHNSON, B. CONNOR. *Methods of Vitamin Determination.* 1948, Burgess Publishing Co., Minneapolis, Minnesota.

13. MCLAREN, BARBARA A., AND HARRINGTON, ALBERT H. Unifine flour. *Baker's Digest,* **24**, 101 (1950).

14. MACRAE, T. F., HUTCHINSON, J. C. D., IRWIN, J. O., BACON, J. S. D., AND MCDOUGALL, E. I. Comparative digestibility of whole meal and white breads and the effect of the degree of fineness of grinding on the former. *J. Hygiene,* **42**, 423 (1942).

15. OSBORNE, T. B., MENDEL, L. B., AND FERRY, E. L. A method for expressing numerically the growth promoting values of proteins. *J. Biol. Chem.,* **37**, 223 (1919).

16. PEASE, GEORGE E., WAHL, TOM, SHOUP, NORMAN H., MCLAREN, BARBARA A., CORBETT, MARY N., AND HARRINGTON, ALBERT H. Unifine flour. Wash. State Inst. of Technol., *Bull.,* **206** (1950).

17. RUBNER, M. *Z. Biol.,* **19**, 45 (1883).

18. WHITNEY, DORIS E., HERREN, HELEN, AND WESTERMAN, BEULAH D. The thiamine and riboflavin content of the grain and flour of certain varieties of Kansas-grown wheat. *Cereal Chem.,* **22**, 90 (1945).

19. WRIGHT, N. C. Digestibility of national wheat meal. *Lancet,* **242**, 165 (1942).

EFFECTS OF VARIOUS PHYSICAL TREATMENTS UPON THE AMYLOCLASTIC SUSCEPTIBILITY OF STARCH

FRANCES E. VOLZ[a] and P. E. RAMSTAD
School of Nutrition, Cornell University, Ithaca, New York

(Manuscript received July 31, 1951)

Starch is a major constituent of all cereal products and many vegetables. The physical treatments involved in such processing operations as baking, canning, freezing, and subsequent storage may alter the starch and thus produce changes in the appearance, texture, and digestibility of these foods.

In nature, starch occurs in discrete, cold-water insoluble, birefringent granules of sizes and shapes characteristic of its source. When these granules are intact, they are quite resistant to attack by amylolytic enzymes. However, modification of the granular structure as a result of mechanical damage or heating in the presence of water results in a greatly increased water imbibing capacity. This is accompanied by increased amyloclastic susceptibility, loss of optical anisotropy, loss of opacity, and increased paste viscosity.

Some starches contain enough of the A-fraction, or straight-chain starch molecules, to form a gel structure as their gelatinized pastes are cooled. As these gels are aged at room temperature they undergo a further change known as retrogradation. This involves an aggregation of molecules which is accompanied by a loss of water-holding capacity and to some extent a reversal of the other changes which occur upon gelatinization. Schoch and French *(11)* have discussed the structure and the aggregation of the A- and B-fractions of starch in detail. Measurements of swelling power and the amount of soluble starch and iodine affinity of the solubles from bread and wheat starch pastes show that both the straight and branched chain starch molecules are involved in the aggregation which occurs during aging of starch pastes. Thus even pastes of waxy maize starch which contains only negligible amounts of the A-fraction exhibit some tendency to retrogradation *(10)*.

Certain treatments, for example, freezing, have been reported to accelerate and accentuate retrogradation. However, Woodruff and MacMasters *(13)* observed that starch pastes frozen at the temperatures of liquid air or solid carbon dioxide did not exhibit as great changes as those frozen at higher temperatures. They considered a transition temperature of −25°C. as the dividing point in the effect of freezing upon the physical characteristics of starch pastes.

Woolrich and Bartlett *(14)* in a discussion of quick freezing of foods emphasized the effects of freezing rate upon changes which may occur in colloidal systems. They point out that perishable foods are colloidal systems in which the external or dispersing phase is an aqueous solution.

[a] Present address: Knudsen Creamery Co., Los Angeles, California.

Freezing causes concentration by removing liquid water from the external phase, thereby decreasing the distance between dispersed particles and bringing about instability of the colloid. Thawing rate as well as freezing rate can be important in this respect. This has been demonstrated in the instance of the colloidal changes occurring in eggs. Moran *(9)* noted that hen eggs maintained normal consistency when frozen in liquid air and rapidly thawed. Slowly frozen or slowly thawed egg yolks acquire a thick, rubbery texture, evidence of the colloidal change which has taken place.

Bachrach and Briggs *(2)* believe the aggregation of starch molecules results from the formation of cross linkages through hydrogen bonds which involve a small number of water molecules. They were able to show that as starch retrogrades water is bound. In the absence of moisture retrogradation does not occur.

Physical changes in a colloidal system can best be described in terms of some property which can be measured quantitatively. Lansky, Kooi, and Schoch *(6)* have expressed the relative retrogradation tendencies of various linear starch fractions in terms of the time required for development of initial turbidity in 1% solutions.

As already pointed out, starch in the form of raw, undamaged granules is not readily susceptible to enzymatic attack. As gelatinization and retrogradation take place enzymatic susceptibility is altered primarily because of the change in availability of the starch surface to the enzyme. Susceptibility to enzymatic attack might be expected to give an indication of the physical state of the starch in terms of an objective measurement.

If starch is digested with an excess of enzyme, the reaction rate will be largely a function of the susceptibility of the starch to hydrolysis. This can be measured by determining the increase in reducing power following digestion for a specific time interval under standardized conditions of temperature, pH, and concentration.

In addition to indicating the magnitude of the physical changes occurring in starches, amyloclastic susceptibility of a starch is in itself important for two reasons: 1, all starches are of value as foods only to the extent to which they are hydrolyzed to sugars in the digestive tract; and 2, many food processing operations involve or are accompanied by a partial enzymatic hydrolysis of starch.

MATERIALS AND METHODS

Commercial potato, corn, and waxy maize starches were used as the starting materials in the experiments reported here. Retrogradation of starch and its reversal are so sensitive to changes in environmental conditions, it was found to be very important to standardize all conditions other than the variables under consideration in each experiment. Temperature of the cooked pastes at the time of sampling, concentration, holding time before sampling, and method of sample preparation were found to be extremely important.

Amyloclastic susceptibility of starch was determined by digestion with Takadiastase[b] in 0.05 *M.* sodium acetate buffer, pH 5.2, at 30°C. for 30 minutes. Enzymatic activity was stopped by the addition of 4% by volume of both 10% sulfuric acid and 12% sodium tungstate solutions. The digestion mixtures were then made to volume and filtered through Whatman No. 4 paper. The reducing capacity, measured by the ferricyanide method *(1)*, was determined on a 5-ml. aliquot of the filtrate. This value was

[b] Manufactured by Parke, Davis and Co., Detroit, Michigan.

then converted to a dry-weight basis and reported as milliequivalents of ferricyanide reduced per 100 g. of dry starch.

Takadiastase was the enzyme selected for these comparisons because of its stability in solution at the digestion temperature. Throughout these studies 100 mg. of enzyme per gram of starch was used.

RESULTS AND DISCUSSION

Mechanical damage. Raw undamaged starch granules are attacked by enzymes in vitro to only a slight extent. For example, raw corn starch when digested by a 1:10 ratio of enzyme to substrate for 30 minutes at 30°C. showed the following increases in reducing power: animal diastase,[c] 260; pancreatin,[d] 100; and Takadiastase, 40. These values represent a very small fraction of the theoretical reducing capacity of 2640 for complete conversion of starch to maltose.

TABLE 1

Effect of ball-milling on the amyloclastic susceptibility of raw starches and retrograded potato starch [f]

	Milling time in hours					
	0	45	71	113	201	230
	E.S.[g]	E.S.	E.S.	E.S.	E.S.	E.S.
Raw corn starch	40	.....	2250	2420	2400	.....
Raw waxy maize starch	80	2130	2330	2400	.....	.....
Raw potato starch	40	.....	2580	2650	.....	.....
Retrograded potato starch	400	.....	2380	2390	.....	2410

[f] A 10% cooked potato starch paste was retrograded by slow freezing, then was dried and pulverized.

[g] E.S. designates "enzymatic susceptibility" expressed in terms of milliequivalents of ferricyanide reduced per 100 g. of dry starch digested.

Mechanical damage causes changes similar to those produced by heating in the presence of water. Extensive damage, such as is accomplished by ball-milling of dry starch, results in a product which will form a paste, has lost its optical anisotropy, and is readily susceptible to attack by enzymes. Lampitt, Fuller, and Goldenberg *(5)* carried out a series of exhaustive experiments on the fractionation of wheat starch by ball-milling. However, their work did not include any studies on the increased susceptibility of the starch to enzymatic attack.

The work reported here was done to determine the effect of ball-milling on the enzymatic susceptibility of starches. Air-dried raw corn starch, waxy maize starch, potato starch, and dried cooked potato starch which had been retrograded by very slow freezing, were milled in porcelain-lined ball-mills. Then 500-g. samples of the starch with 5360 g. of Borundum* balls (240 balls) were milled up to 230 hours.

At intervals, samples were withdrawn and enzymatic susceptibilities determined. To obtain a uniform aqueous dispersion of the milled starch it was necessary to add 4 ml. of alcohol to a 1-g. sample before adding the buffer. The results are given in Table 1.

[c] Diastase (animal) 1-50-5 min. Manufactured by Nutritional Biochemicals Corp., Cleveland, Ohio.

[d] Pancreatin, U.S.P. Manufactured by Nutritional Biochemicals Corp., Cleveland, Ohio.

* Borundum grinding media. Manufactured by The U. S. Stoneware Co., Akron, Ohio.

Ball-milling of starch results in granule degradation and greatly increases enzymatic susceptibility. Retrograded potato starch did not reach as high a level of mechanical breakdown as the raw potato starch even after 230 hours under these milling conditions. There is variation in the rate of disintegration of the raw starches. Potato starch which gelatinizes at a lower temperature than the other raw starches breaks down more rapidly during milling. This difference is probably due to the larger granule size which facilitates contact of the balls and the granules, thereby hastening mechanical degradation.

Preliminary trials using a less efficient mill produced much slower changes. It is important to keep in mind the variations which are possible in the ball-milling treatment, such as size of mill, quantity of charge, ratio of charge to balls, and speed of rotation. All of these factors influence the rate of milling. Therefore, the data reported here cannot be compared directly with the results obtained by investigators using different milling conditions.

Heating. As noted before, raw starch is attacked by enzymes in vitro to only a slight extent.

A sharp increase in enzymatic susceptibility occurs when starch gelatinizes. To show the magnitude of this increased susceptibility, a series of starch samples were heated in water before digestion with Takadiastase.

First, 1-g. samples of corn, potato, and waxy maize starches were suspended in 100 ml. of 0.05 *M.* sodium acetate buffer, pH 5.2, and heated at 50°, 60°, 70°, 75°C., and in an autoclave at 15 p.s.i.g. for 10 minutes. The starch suspensions were then cooled to 30°C. and 100 mg. of Takadiastase were added to each. The general procedure for determination of amyloclastic susceptibility was then followed. The results of this comparison are shown in Table 2.

TABLE 2

Effect of heating in water on the enzymatic susceptibility of starches

Pretreatment		Type of starch used		
Temperature	Time	Corn	Potato	Waxy maize
		E.S.[1]	E.S.	E.S.
°C.	*minutes*			
30	10	40	60	80
50	10	.....	15	.....
60	10	100	2100	170
70	10	2250	2530	1960
75	10	2290	.....	2360
Autoclave, 15 p.s.i.g.	10	2280	2530	2400

[1] See footnote [2], Table 1, for meaning of E.S.

The enzymatic susceptibility increased sharply at the gelatinization temperature of the starch: corn, 70°C.; potato, 60°C.; and waxy maize starch, 70°C. The values observed at lower temperatures are probably accounted for by the presence of damaged granules which are readily attacked by enzymes.

Concentration. Difficulty in preparing homogeneous starch pastes of high concentration is caused by unequal distribution of the limited moisture as the heating progresses. Schoch and French *(11)* prepared 50% starch pastes by slowly heating the starch suspensions to 100°C. over a period of 30 minutes. The paste was then heated in a boiling water bath for an additional 30 minutes. In the work reported here, the difficulty inherent in the preparation of concentrated pastes was overcome by cooking in an electronic oven[h] which gave uniform and thorough heating throughout the entire paste very quickly. Then 20 and 50% corn starch pastes were compared with 10% pastes using this cooking procedure. Results are given in Table 3. Because of the difficulty encountered in sampling and suspending such dense pastes, the samples were freeze-dried and ground in a Wiley mill to permit uniform sampling. The freeze-drying process causes a slight alteration in enzymatic susceptibility as reported later in this paper.

TABLE 3

Effect of concentration on the enzymatic susceptibility of corn starch pastes heated in an electronic oven

Series[i]	Treatment	Corn starch concentration, %		
		10	20	50
		E.S.[k]	E.S.	E.S.
1	Fresh, lyophilized	2130	2100	580
2	Fresh, lyophilized	2080	1960	560
3	24 hr. refrigerated, lyophilized	.....	1540	290

[i] Series 1 and 2. Cooked for 3-15 sec. intervals, stirring after each interval. Pastes cooled to room temperature before lyophilization. Series 3. Cooked for 6-15 sec. intervals, stirring after each interval. Pastes refrigerated for 24 hours before lyophilization.
[k] See footnote [g], Table 1, for meaning of E.S.

Limiting the water available for gelatinization lowers the enzymatic susceptibility of the resulting paste. This was especially evident at the 50% concentration. This limitation of swelling and the resulting low enzymatic susceptibility indicates that the 50% paste has changed only slightly from the raw starch.

Refrigerated storage. Temperature has an important effect upon the rate of starch retrogradation. Katz *(4)* found that bread crumb remained fresh at temperatures of 80°C., but staled very rapidly at 0°C. The rate of decrease in enzymatic susceptibility of a refrigerated starch paste was shown by the following experiment.

Portions of a gelatinized 10% corn starch paste were placed in large test tubes and plugged with cotton. After 20 minutes of autoclaving at 15 p.s.i.g. these samples were stored at 4°C. Amyloclastic susceptibility was determined at intervals over a month's time, with the results shown in Table 4. At the end of 31 days the starch was 40% retrograded as measured by enzymatic susceptibility.

[h] Radarange. Manufactured by the Raytheon Sales Corporation, Radarange Division, Waltham, Mass.

Freeze-drying. Freeze-drying was observed to have a dual effect upon the enzymatic susceptibility of starch pastes. Fresh pastes may be frozen and their enzymatic susceptibility lowered. But another change takes place during drying, the susceptibility of some retrograded pastes appears to increase under certain drying conditions. To show the extent of improvement which can occur, portions of the starch pastes used in the preceding experiment were freeze-dried at each sampling period. The dried samples were ground in a Wiley mill and their enzymatic susceptibilities were determined. Figure 1 compares values obtained before and after freeze-drying. To determine whether the retrogradation which occurs during freezing of a starch paste is also reversed by lyophilization, 1% corn starch and potato starch pastes were compared before and after drying. The results are shown in Table 5.

TABLE 4

Effect of aging corn starch paste at refrigerator temperatures upon enzymatic susceptibility

Hours at 4°C.	Enzymatic susceptibility[1]
0	2080
20	1950
67	1850
144	1750
240	1600
744	1250

[1] See footnote [e], Table 1, for meaning of E.S.

TABLE 5

Effect of freeze-drying on the enzymatic susceptibility of frozen starch pastes

Starch	Treatment of the starch paste		
	None	Frozen, thawed	Frozen, lyophilized
	E.S.[m]	E.S.	E.S.
Corn	2240	1570	1870
Potato	2470	1590	1890

[m] See footnote [e], Table 1, for meaning of E.S.

The data in Figure 1 as well as in Table 5 indicate that the drying process may break some of the weak bonds formed during retrogradation of starch. Figure 1 shows that in the 0-, 20-, and 67-hour samples, lyophilization (which includes freezing) resulted in more starch aggregation than had been caused by aging alone. After this period (at 144, 240, and 744 hours), weak bonds continued to be formed during refrigerated storage, but were broken by subsequent processing, thereby increasing the enzymatic susceptibility over that of the undried paste.

Freezing rate. The effect of freezing rate on the quality of a foodstuff depends upon the nature of the food. No distinct or measurable chemical differences between slow and fast frozen food have been reported, in spite of exaggerated physical changes *(7)*. Woodruff and MacMasters

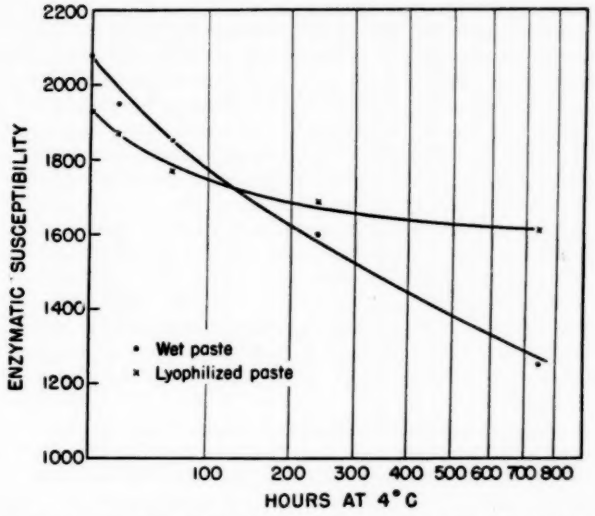


Figure 1. A comparison of the effects of freeze-drying (lyophilization) upon the amyloclastic susceptibilities of starch pastes aged at 4° C.

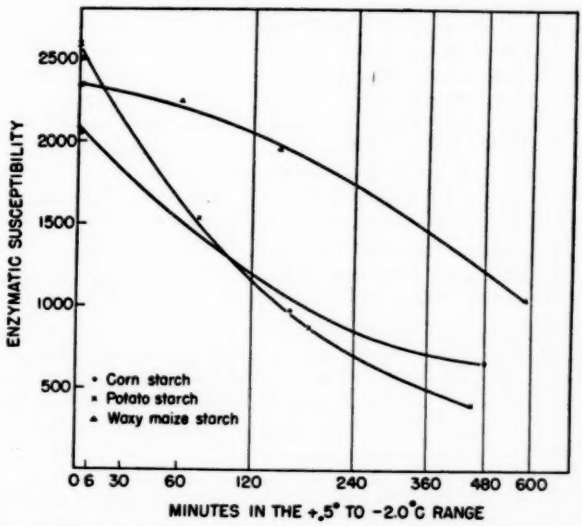


Figure 2. Effect of freezing rate upon the enzymatic susceptibility of 10% pastes of 3 starches.

(13) reported gross physical changes in starch pastes frozen under different conditions. Therefore, the amyloclastic susceptibility of frozen starch pastes has been investigated to determine the effect of freezing rate on gelatinized starch. The 10% corn, potato, and waxy maize starch pastes were cooked for 20 minutes in an autoclave at 15 p.s.i.g. After partial cooking, 50-g. portions of the pastes were poured into paper cups. Thermocouples were placed in the centers of representative samples, and the rate of freezing was followed with a recording potentiometer.[n] The range of +.5° to −2.0° C. was selected for calculation of the freezing rates, since this temperature range seemed to be a clearly defined plateau during ice crystal formation. The frozen pastes were thawed rapidly by slicing finely with a sharp knife; less than 10 minutes was required to thaw the 50-g. samples. The thawed pastes were stirred thoroughly before weighing 10-g. portions for digestion. Since the thawing time was constant, any difference in the enzymatic susceptibility of the pastes can be attributed to the differences in freezing rates. A representative series of samples is shown in Figure 2.

These data indicate that the changes in starch commonly associated with freezing actually occur just prior to actual freezing and that the extent of retrogradation is a function of the length of exposure to temperatures in the freezing range. Bonding between molecules occurs most rapidly during the period of ice crystal formation. Prolongation of this period results in extensive bonding. Very rapid freezing of starch pastes by immersion in a dry-ice alcohol mixture resulted in such rapid ice crystal formation (less than 1 minute) that the amyloclastic susceptibility was altered only slightly. As the freezing rate was slowed down the susceptibility decreased progressively. Although the waxy maize starch showed less change in susceptibility to enzymatic attack, the freezing rate effects resembled those of corn and potato starch. The change in enzymatic susceptibility of waxy maize starch was more gradual than that of corn and potato starches. This was not surprising since bonding of the A-fraction takes place more readily than that of the B-fraction. After 3 hours in the freezing range more than half of the corn and potato starch molecules are in aggregates which are not readily attacked by enzymes. On the other hand, waxy maize starch paste has lost only 15% of its potential susceptibility.

The texture of the very slowly frozen waxy maize starch pastes was soft and spongy, whereas the other 2 starches formed tough, fibrous masses when frozen slowly. Water could be expressed from all the slowly frozen pastes, even from the waxy maize starch.

Thawing rate. Since the change in enzymatic susceptibility of frozen starch pastes appears to be a function of time in the temperature range where ice crystals are forming, it seemed possible that thawing time might have a similar effect. Therefore, a series of samples was prepared to study thawing rates.

---

[n] Manufactured by The Brown Instrument Company, a Division of Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.

First, 10% starch pastes were prepared and handled in the manner described in the previous section. The cooked pastes were placed in a freezer having an air temperature of −29°C. The length of time in the +.5° to −2.0°C. range was 60 to 85 minutes as indicated in Table 6.

Thermocouples were placed in several samples before freezing. After freezing was complete, the samples were thawed at different rates. When thawing was complete the samples were thoroughly mixed with an electric stirrer. The results of the amyloclastic susceptibility determinations are given in Table 6.

The effect of thawing rate was analogous to that of freezing rate. The longer the period of time in the −2.0°C. to +.5°C. range the lower the amyloclastic susceptibility of the thawed paste. The 10-minute thawing time data (corn, 1480; potato, 1520; waxy maize starch, 2240) represent essentially the change occurring during freezing. The other figures represent the combination of both freezing and thawing effects on molecular aggregation. A correlation coefficient of −0.79 between enzymatic susceptibility and time in the temperature range +.5° to −2.0°C. was calculated on the basis of 21 observations (freezing and thawing times combined).

Reheating frozen pastes. Since frozen foods may be heated just before use, the effect of reheating upon the enzymatic susceptibility of frozen pastes was also investigated.

TABLE 6

Effect of thawing rate on the amyloclastic susceptibility of 10% starch pastes

Starch	Thawing time −2.0° to +.5°C.	Enzymatic susceptibility[a]
	minutes	
Corn	10	1480
	40	860
	215	750
	310	520
Potato	10	1520
	75	940
	180	620
	215	470
Waxy maize	10	2240
	40	2140
	100	1950

[a] See footnote [a], Table 1, for meaning of E.S.

First, 1 and 10% starch pastes were partially gelatinized by heating in a boiling water bath, then were autoclaved for 20 minutes at 15 p.s.i.g. Then 50-g. portions of the 10% pastes were frozen in paper cups. Then 20-ml. aliquots of the 1% starches were pipetted directly into 125-ml. Erlenmeyer flasks. These samples were frozen very slowly in an insulated box, in a −18°C. freezer. The 10% pastes were thawed before an electric fan, the 1% starch samples were thawed in a 30°C. water bath. After thawing, some samples were placed in a 55°C. water bath and held for 10

minutes after the paste had reached 55°C. Other samples were autoclaved for 10 minutes after thawing. Results are given in Table 7.

Reheating of frozen starch pastes reverses most of the retrogradation changes in the starch as measured by enzymatic digestion. A reheating temperature of 55°C. for 10 minutes effected almost as complete a reversal of the retrogradation as autoclaving. However, even after autoclaving for 10 minutes at 15 p.s.i.g. the reversal was not complete for any sample except for waxy maize starch. These observations indicate that a portion of the A-fraction of corn and potato starch is irreversibly bound during the freezing process, and that waxy maize starch does not undergo this irreversible change. The results of this experiment agree with the conclusions of Longrée *(8)* that frozen cooked potatoes had to be reheated to a definite temperature (50.5° — 56°C.) in order to regain their original texture.

Frozen storage. An experiment was carried out to find out if frozen starch pastes might undergo a change during storage which would alter their amyloclastic susceptibility. The 10% pastes of corn and waxy maize starch were prepared by autoclaving at 15 p.s.i.g. for 20 minutes, freezing quickly and storing at —18°C. Samples were withdrawn at intervals during an 8-month storage period and their enzymatic susceptibilities determined. No clear-cut change was evident in either starch during this time.

TABLE 7

Effect of reheating upon the enzymatic susceptibility of starch pastes that have been frozen slowly

	10% corn	1% corn	1% potato	1% waxy maize
	E.S.[p]	E.S.	E.S.	E.S.
Initial, before freezing	2110	2230	2360	2280
Frozen, thawed	640	1470	1330	2200
Frozen, reheated to 55°C.	1970	2050	1990	2240
Frozen, reheated by autoclaving, 10 min. at 15 p.s.i.g.	2000	2100	2210	2270

[p] See footnote [5], Table 1, for meaning of E.S.

Starch in foods. The experimental work described thus far was done on simple starch buffer solution systems. Changes in the enzymatic susceptibility of the starch constituent of various foods can also be observed.

In a previous report *(12)* the authors observed a drop in the amyloclastic susceptibility of bread crumb from 1060 1 hour after baking to 220 after 72 hours. Addition of polyoxyethylene monostearate or fatty acid monoglycerides decreased the rate at which bread crumb became firmer with age but did not affect the change in enzymatic susceptibility.

In another experiment a potato autoclaved at 15 p.s.i.g. for 15 minutes showed an amyloclastic susceptibility (on a dry matter basis) of 1800. When stored overnight in a refrigerator this dropped to 1200. Slow freez-

ing caused a further decrease to 230. Upon thawing and reheating to an internal temperature of 58°C. an increase to 1410 was observed.

The method might be used in a similar manner for following changes in the starch portion of many other foods.

SUMMARY AND CONCLUSIONS

A method for measuring the susceptibility of starch to attack by Takadiastase was used to observe the extent of gelatinization and retrogradation caused by various physical treatments and storage conditions.

Marked increases in amyloclastic susceptibility were caused by physical damage to starch granules and by heating in the presence of water to gelatinization temperatures. Some increase resulted from drying of gelatinized pastes under certain conditions. At high ratios of starch to water, lack of sufficient water for complete gelatinization limited the extent of enzymatic susceptibility produced by heating.

Pastes of gelatinized starch showed varying tendencies to loss of amyloclastic susceptibility as a consequence of retrogradation depending upon the type of starch and the treatments they received. Waxy maize starch showed less change than did either corn or potato starches. Loss of enzymatic susceptibility following freezing and thawing of pastes was found to be inversely proportional to the rates at which the freezing and thawing processes took place. That is, rapid freezing and thawing caused the least change. Storage at —18°C. for 8 months was without discernible effect.

The procedure used is adaptable, with slight modification, to the study of changes in the starch fraction of bread, potatoes, and other foods.

LITERATURE CITED[1]

1. American Association of Cereal Chemists. *Cereal Laboratory Methods.* 5th Ed., 1947, St. Paul, Minnesota.

2. Bachrach, Howard L., and Briggs, D. R. Studies on bread staling. II. Water relationships during staling of bread crumb and the retrogradation of starch. *Cereal Chem.*, **24**, 492 (1947).

3. Bailey, L. H. The use of certain constituents in breadbaking with particular reference to the problem of staling. *Cereal Chem.*, **9**, 65 (1932).

4. Katz, J. R. Gelatinization and retrogradation of starch in relation to the problem of bread staling. Chapter in: *A Comprehensive Survey of Starch Chemistry.* Edited by R. P. Walton, 1928, The Chemical Catalog Co., New York, N. Y.

5. Lampitt, L. H., Fuller, C. H. F., and Goldenberg, N. The fractionation of wheat starch. II. The reducing powers of starch fractions. VII. Retrogradation of starch solutions. *J. Soc. Chem. Ind. (London)*, **60**, 25, 175 (1941).

6. Lansky, Sylvia, Kooi, Mary, and Schoch, T. J. Properties of the fractions and linear subfractions from various starches. *J. Am. Chem. Soc.*, **71**, 4066 (1949).

7. Lee, F. A., Gortner, W. A., and Whitcombe, Joanne. Effect of freezing rate on vegetables. *Ind. Eng. Chem.*, **38**, 341 (1946).

8. Longrée, Karla. Quality problems in cooked frozen potatoes. *Food Technol.*, **4**, 98 (1950).

9. Moran, T. Effect of low temperatures on hen eggs. *Proc. Royal Soc. (London), B*, **98**, 436 (1925).

10. Noznick, P. P., Merritt, P. P., and Geddes, W. F. Staling studies on breads containing waxy maize starch. *Cereal Chem.*, **23**, 297 (1946).

11. Schoch, T. J., and French, D. Studies on bread staling. I. The role of starch. *Cereal Chem.*, **24**, 231 (1947).

12. Volz, Frances E., and Ramstad, P. E. Effect of emulsifiers on the enzyme susceptibility of starch during staling of bread. *Cereal Chem.*, **28**, 118 (1951).

13. Woodruff, Sybil, and MacMasters, Majel M. Gelatinization and retrogradation changes in corn and wheat starches shown by photomicrographs. Univ. Illinois Agr. Exp. Sta., *Bull.*, 445 (1938).

14. Woolrich, W. R., and Bartlett, L. H. Quick and flash freezing of foods. *Mech. Eng.*, **64**, 647 (1942).

NEW EDITION OF "INFORMATION AND SUGGESTIONS FOR AUTHORS" PUBLISHED

A new and expanded edition of the pamphlet "Information and suggestions for authors" has been published for those writing technical contributions for *Food Technology* and *Food Research*.

The pamphlet discusses the respective scopes of the two journals and the type of articles suitable for them. A detailed outline is presented of the customary organization of technical papers followed by statements concerning the preferred styles and methods of preparing manuscripts. A discussion of various details like units of measurements and their abbreviations, use of footnotes and literature citations, preparation of tables and figures, etc. follows.

In the following chapter the customary methods of correcting printer's proofs are described. Here a list is given indicating the more common corrections. Instructions for the ordering of reprints follow.

Prospective authors are urged to consult this pamphlet which will be sent free of charge upon a request addressed to the editorial office.

Authorship in *Food Technology* and *Food Research* is not restricted to members of the Institute of Food Technologists.

DIFCO

PEPTONES AND HYDROLYSATES
for Microbiological Culture Media

Difco peptones and hydrolysates are prepared to meet the diversified nutritional and biochemical requirements of microorganisms. A single peptone cannot provide the essential nitrogenous nutriments for all cultural and metabolic processes. Difco peptones and hydrolysates have been developed to meet the various requirements for microbiological procedures including growth, preparation of toxin and vaccines, and for the study of microbial metabolism.

BACTO-PEPTONE is most widely used in the preparation of routine culture media. It is rich in readily available forms of nitrogen, and in a one per cent solution it is sparklingly clear with a reaction of pH 7.0. Bacto-Peptone is specified in the formulae recommended in "Standard Methods of Water Analysis" of the American Public Health Association.

BACTO-TRYPTOSE is a peptone which was originally developed for cultivation of the *Brucella*. A two per cent solution, as the sole source of nitrogen, is an excellent substitute for the meat infusion generally employed for propagation of the *Streptococci, Pneumococci, Meningococci* and other discriminative bacteria.

PROTEOSE PEPTONE is universally used in the preparation of media for elaboration of diphtheria toxin. It is also an excellent nutriment for use in media designed for the production of other bacterial toxins such as those of scarlet fever and botulinus.

PROTEOSE PEPTONE NO. 3 is particularly suitable for use in culture media employed for isolation and propagation of *Neisseria gonorrhoeae* and *Corynebacterium diphtheriae*.

NEOPEPTONE is especially recommended as an ingredient of culture media for isolation and study of *Streptococci, Pneumococci* and Fungi. Media prepared with Neopeptone are most satisfactory for cultivation of *Streptococci* in the smooth or mucoid phase. Fungi produce typical characteristic colonies on solid media prepared with Neopeptone.

BACTO-TRYPTONE is especially adapted for the elaboration of Indol and for use in media to detect Hydrogen Sulfide production. This peptone is recommended in "Standard Methods for the Examination of Dairy Products" of the American Public Health Association for the medium used in milk counts.

BACTO-CASITONE is a pancreatic digest of casein recommended for use in the preparation of media for sterility testing according to standard procedures, and for the preparation of media requiring an enzymatic hydrolyzed casein. Bacto-Casitone can be used for the detection of Indol production by microorganisms, since it has a high tryptophane content.

BACTO-CASAMINO ACIDS is an acid hydrolyzed casein originally prepared for the production of diphtheria toxin in peptone free media, and since adapted for other toxins and vaccines. Iron, copper, and other heavy metals have been removed from Bacto-Casamino Acids. This hydrolysate is excellently suited for nutritional studies of microorganisms.

BACTO-CASAMINO ACIDS TECHNICAL is an acid hydrolyzed casein recommended for use in media where amino acids mixture are required as a source of nitrogen, and the sodium chloride content is not a limiting factor. Bacto-Casamino Acids Technical is of particular value in nutritional studies of bacteria.

BACTO-VITAMIN FREE CASAMINO ACIDS is acid hydrolyzed casein, free from vitamins. It is recommended for the preparation of media for microbiological assay of vitamins and tryptophane. This hydrolysate is a valuable aid in studies of growth requirements of microorganisms.

Specify "DIFCO"

DIFCO LABORATORIES

DETROIT 1, MICHIGAN